Accelerating COVID-19 Therapeutic Interventions and Vaccines (ACTIV) Post-Acute Sequelae of SARS-CoV-2 (PASC)/Long COVID Seminar Series Whitepaper

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All seminar recordings are available to watch here: ACTIV Seminar Series: Post-Acute Sequelae of SARS-CoV-2.

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Introduction
The National Institutes of Health (NIH) developed the Accelerating COVID-19 Therapeutic Interventions and Vaccines (ACTIV) public-private partnership to coordinate a research strategy for prioritizing and speeding development of the most promising treatments and vaccines, including treatments for post-acute sequelae of SARS-CoV-2 (PASC). To assist with this effort, ACTIV convened the Pre-Clinical working group to create preliminary inventories of the most relevant and promising models and assays for understanding COVID-19 and PASC and to develop criteria for rapidly prioritizing clinical treatments.

The earliest reports of what we now recognize as PASC, or long COVID, are difficult to pinpoint with precision. The British Medical Journal has attributed the first use of the term to a Twitter hashtag used by Dr. Elisa Perego on May 20, 2020 to describe her own relapsing and remitting symptoms that persisted beyond the recognized acute COVID-19 period. Based on the current World Health Organization (WHO) working case definition of post-COVID-19 conditions — symptoms present typically 3 months beyond an acute COVID-19 diagnosis and that have persisted for 2 months in the absence of an alternative diagnosis — May 2020 would have been close to the earliest possible point to recognize what we now call long COVID. Participants in the COVID-19 Core Outcomes Set workshops convened in April 2020 gave voice to significant symptom burden that some COVID-19 survivors were experiencing beyond the acute illness period, and concerns about longer term post-viral conditions were raised even at that early date. So, it could rightly be said that we’ve known about long COVID almost as long as we’ve known about COVID-19 itself.

Two years after the recognition of long COVID, much has been learned and many new questions raised. The first and perhaps most challenging aspect of long COVID that has become clearer in those two years is that long COVID is quite protean in its manifestations. This is in rather sharp contrast to acute COVID-19. Acute COVID-19 is a disease with a distinct cause — infection with the SARS-CoV-2 virus — that can be ascertained with a variety of molecular tests (e.g., PCR, viral antigen testing, virus-specific antibody testing) and that has a known spectrum of clinical manifestations that are predominantly characterized by viral pneumonia and associated symptoms, as well as some more virus-specific manifestations (e.g., anosmia and dysgeusia prominent in the pre-Omicron epochs of the pandemic). Thus, diagnostic ascertainment is fairly well established, even with home-based technologies.

Long COVID, on the other hand, has virtually none of these features. The number of organ systems known and/or suspected to be affected as part of the spectrum of long COVID is surprisingly long – central nervous system, peripheral nervous system, heart, lungs, GI tract (including dysbiosis of the gut microbiome), liver, kidneys, pancreas, skeletal muscle, joints and connective tissues, skin, immune system, and others. This has made basic efforts to define the syndrome of long COVID surprisingly difficult, with many working definitions having been generated but no single consensus definition available for definitive diagnosis. Similarly, there are no biomarkers, laboratory tests, imaging studies, or other diagnostic instruments that have been validated for diagnosing long COVID. Indeed, even the discussions about the “COVID-19” part of long COVID are more challenging that what might at first be obvious. Many people infected early in the pandemic had no definitive testing for SARS-CoV-2 because it was not widely available. It is recognized that asymptomatic infection with SARS-CoV-2 definitely happens, and that this can lead to post-acute sequelae of SARS-CoV-2 infection (PASC) in at least some people.
The understanding of the pathobiology of acute COVID-19 and the identification of potentially useful intervention strategies were greatly accelerated by the availability of multiple robust preclinical models of acute SARS-CoV-2 infection. There was and is no single ideal preclinical model, but taken together the models have been indispensable to the successes of the first phases of the global pandemic response. This led to early discussions about how this same strategy might be applied to long COVID/PASC. However, this led very quickly to a fundamental problem, namely: If you can’t readily identify or define long COVID/PASC in humans, it becomes prohibitively difficult to develop a preclinical model because, simply put, you don’t really know what you’re modeling.

This is not a problem unique to the SARS-CoV-2/COVID-19 pandemic. There are a number of chronic diseases for which preclinical models are imperfect representations of the human disease. Two examples from the world of pulmonary medicine are the bleomycin model of pulmonary fibrosis and the monocrotaline model of pulmonary hypertension. These are both models in which a toxin is administered to rodents, and the resulting tissue changes and pathophysiology resemble at least some aspects of the human disease being modeled. Bleomycin will produce fibrosis in rodent lungs, and monocrotaline will produce pulmonary hypertension. To say that either of these are full and faithful reproductions of the human disease they’re meant to model would be incorrect. But, it would also be incorrect to say that these models have been useless, because each has provided important information that has informed better understanding of the human diseases.

Even with imperfect preclinical models, though, one still needs to have a starting point to know something about what one is trying to model. A usual approach for developing a new preclinical model would be to assemble experts in the disease that one is trying to model. That group would then discuss the key salient features of the disease/condition, discuss how those salient features might be best represented in known model systems, and delineate the pros and cons of the best available model systems. This process was employed to great effect early in the COVID-19 pandemic, with field guides for studying acute SARS-CoV-2 infection in small animal models and in nonhuman primate models providing invaluable perspectives.

Here again, though, long COVID/PASC has presented a major hurdle. At the time this seminar series was being organized, there were no long COVID experts, and some have said that is still true even now. Nonetheless, features of long COVID/PASC have emerged with increasing clarity through ongoing discussions amongst patients, caregivers, advocates, clinicians, researchers, and many others. This seminar series sought to try to capture and distill at least a portion of the collective knowledge regarding long COVID/PASC to better identify knowledge gaps and opportunities to accelerate progress toward better diagnosis, treatment, and prevention of this puzzling and debilitating syndrome.

The initial approach for this seminar series was to assemble a roster of speakers who could help address three key questions:

1. What are the major clinical features of PASC/long COVID recognized by patients, caregivers, and clinicians that should inform pathobiological mechanistic investigations, preclinical model development, and future clinical studies?
2. What facets of PASC/long COVID might be ready for focused attention but were not necessarily top-of-mind in long COVID discussions as of August-September 2021?
3. Where do opportunities exist for more rapidly translating PASC/long COVID findings into clinical studies and ultimately into informing clinical practice?

Seven speakers were identified and invited, and all very graciously accepted. The interest and participation in the seminar series from multiple sectors of the community – scientists, clinicians, patients, citizen-scientists, government, regulatory, and many others – was immediate and well beyond what was anticipated. People were engaged both during the live webinars and through the recorded presentations. Given the level of interest, the size of the topic of PASC/long COVID, and the rapidly evolving body of knowledge, the seminar series was extended to include additional speakers and topics that were still oriented around the three key questions above.

IMPORTANT NOTE REGARDING THE CHANGING NATURE OF THE PANDEMIC

The nature of the SARS-CoV-2/COVID-19 pandemic has changed almost too fast for the scientific literature to keep pace. There are instances where even the most efficient peer review processes have not been able to outpace the emergence of new viral variants, for example. In addition to the ongoing emergence of new viral variants around the globe, the addition of new vaccines and vaccination practices, development of new therapeutics, changing testing options and practices, changing opportunities for repeat infection with SARS-CoV-2, and the changing clinical picture of COVID-19 and of PASC/long COVID all contribute to the shifting complexity over time.

As such, readers should be cognizant of the fact that the points and issues discussed by the contributors to this seminar series reflect the state of knowledge at the time of their presentation. Most or all of the main points raised by the contributors are at least as relevant now as they were when the presentations and discussions happened. However, it must be noted that some changes and refinements in the details may have happened in the interim, and this will continue to be the case as we learn more about how to understand and treat long COVID.

Discussion

Several themes emerged from the seminar series as a whole and the data and ideas presented and discussed. All of the major themes are still very much active areas of investigation, with publications and preprints coming online almost daily. Many of these overarching themes will soon be practically interrogated as interventional trials for PASC/long COVID launch, while simultaneously being refined and focused through deeper mechanistic investigations. It should be noted that none of the hypotheses discussed below are mutually exclusive of each other, and there are many plausible options for complex interrelationships between them.

Inflammation, Autoimmunity, and Immune “Training”

Multiple presenters discussed the role of the immune response to SARS-CoV-2 infection as being central to initiating and maintaining the post-acute sequelae of infection. Ongoing and/or aberrant inflammatory activation has emerged as a high priority hypothesis for what is driving PASC/long COVID in some people. The persistent inflammation hypothesis builds on the evidence for the inflammatory response playing a central role in the pathogenesis of severe acute COVID-19 coupled with the finding in several studies that
severity of acute COVID-19 is associated with risk for developing PASC/long COVID. Persistent inflammation could help explain the variability of PASC/long COVID manifestations - through different types of inflammatory activation, responses to different viral variants, and complex interactions between these and other factors such as prior immunizing events (vaccination or prior infection). Persistent inflammatory activation could also help explain the multisystem nature of PASC/long COVID, some of the associations noted between acute COVID-19 and PASC/long COVID, and potentially other trends noted in PASC studies such as age and sex differences. Finally, persistent inflammatory activation could well be working in concert with other hypothesized mechanisms for PASC/long COVID pathogenesis.

A companion hypothesis that has emerged for PASC/long COVID pathogenesis is that of activation of an autoimmune response by SARS-CoV-2 in some individuals. This autoimmunity is thought to manifest as either a new maladaptive immune response to previously tolerated self-antigens, or as an immune response against neoantigens formed as a result of SARS-CoV-2 infection and the acute response to infection. There is ongoing debate about how prominent a role autoimmunity may play in the onset, persistence, and progression of PASC/long COVID, with some studies strongly implicating autoimmunity whereas others have found less supportive evidence for an ongoing autoimmune response as a key pathogenic mechanism in long COVID.

**Viral Persistence or Reactivation**

A hypothesis regarding PASC/long COVID pathogenesis that has garnered a great deal of attention and for which there is fairly strong supportive data is the viral persistence hypothesis. Several published studies have shown that some sort of viral material from SARS-CoV-2 – viral genomic material, viral proteins, degradation products of viral particles – is detectable in a subset of COVID-19 survivors’ blood, body fluids, or tissues for months after clinical resolution of COVID-19. Some of these studies have gone on to correlate PASC/long COVID symptoms with the persistence of detectable viral material.

The viral persistence hypothesis has a number of conceptual features that make it attractive as a hypothesis for PASC/long COVID pathogenesis. The idea of viral reservoirs or viral sanctuary sites is well established for a number of other human pathogenic viruses, including human immunodeficiency virus (HIV) and varicella-zoster virus (VZV). For SARS-CoV-2, the existence of tissue viral reservoirs would be consistent with reports of postmortem detection of virus in multiple tissues months after symptom onset and could also be consistent with the increasing recognition of “rebound.” It is not at all difficult to envision how ongoing presence of virus or viral protein could be a source of ongoing inflammation, tissue injury, and potentially symptoms. Further, depending on which tissue(s) harbor virus in any given individual, viral persistence could potentially help to explain the protean clinical manifestations of PASC/long COVID. Finally, differences in time course of viral clearance and/or viral flares from reservoirs between people might partially explain the observed temporal variations in PASC/long COVID symptoms and manifestations.

Related to the viral persistence hypothesis, there is some evidence to suggest that reactivation of previous viral infections may play a role in PASC/long COVID pathogenesis. For example, reactivation of latent Epstein-Barr virus has been associated with PASC/long COVID symptoms. Epstein-Barr virus reactivation has also been previously implicated in the pathogenesis of multiple sclerosis, a chronic neurologic disease that is very protean in its manifestations and with unclear etiologies. Like the hypothesis of persistence
of SARS-CoV-2 as a driver of PASC/long COVID, reactivation of specific viruses or even other latent pathogens represent an attractive hypothesis both in terms of biologic plausibility and in terms of potential interventions targeting the particular pathogen in question.

**Metabolic and Endocrine Disruption**

Diabetes mellitus was recognized early on as a risk factor for more severe acute COVID-19. What has become clearer over time is that there is likely a subset of COVID-19 survivors that exhibit new onset diabetes mellitus following their acute COVID-19 episode. It is not yet apparent whether this subgroup is predominantly people who had pre-existing impaired glucose control that was exacerbated or accelerated by SARS-CoV-2 infection, or whether new post-COVID-19 diabetes is primarily in people who had no evidence for or significant risk factors for diabetes pre-COVID-19. Similarly, it is not yet clear what the clinical trajectory of new onset post-COVID-19 diabetes will be.

Diabetes is just one example of multiple metabolic and endocrine disruptions that have been observed in the post-COVID-19 period, whether in association with PASC/long COVID symptoms or more broadly. It has been recently reported that people with PASC/long COVID exhibit measurably lower serum cortisol levels than people who do not have long COVID.\(^5\) The gut microbiome has been shown to exhibit dysbiosis in the post-COVID-19 period, with proposed linkages to a number of different symptom manifestations of PASC/long COVID. Metabolic alterations may contribute to long COVID pathogenesis even at the molecular level, with changes in mitochondrial function and intermediary metabolism linked to PASC or PASC-like phenotypes.\(^6\)

It is now evident that SARS-CoV-2 directly infects the pancreas and persistent infection is likely an important factor in the pathogenesis of COVID-19/PASC associated type-2 diabetes. Furthermore, immune impairment in the setting of diabetes likely favors the persistence of the viral reservoir within the pancreas which may further induce the onset or exacerbate pre-existing type-2 diabetes.

**Coagulopathy and Endothelial Dysfunction**

One of the earliest features of acute COVID-19 that clinicians noted as being markedly different from other severe viral pneumonias was the pronounced coagulopathy exhibited by many of the sickest COVID-19 patients in the early first wave of the pandemic. Evidence for both larger vessel clots as well as microthrombi in acute COVID-19 drove several trials of anticoagulation/antithrombotic therapies, though demonstrating benefit in those trials proved challenging. Nonetheless, the possibility of “microclots” as being a driver of PASC/long COVID is a source of ongoing debate.

Related to the microthrombi hypothesis is the hypothesis that widespread endothelial dysfunction is a key driver of PASC/long COVID. This is discussed as a range of endothelial dysfunction, from biochemical and cellular-level alterations to endotheliopathy\(^7\) to inflammatory endotheliitis to endothelial cell death leading to capillary rarefaction or denuding of endothelial surfaces. There is even evidence that SARS-CoV-2 has the capacity to infect\(^8\) endothelial cells directly.

Like several of the other hypotheses for PASC/long COVID pathogenesis, the endothelial dysfunction +/- microthrombi hypothesis has some features that make it appealing as a testable idea. Differential effects on specific vascular beds could account for the variable symptoms and tissue manifestations of PASC/long COVID.
COVID. Differences in timing of viral clearance from endothelial cells and/or in timing of endothelial repair processes could help explain the temporal variability of PASC/long COVID.

**Other Post-Viral/Post-Acute Infection Syndromes**

Though SARS-CoV-2 is a new viral pathogen, the phenomenon of complex and persistent clinical syndromes occurring after acute infectious illnesses is not new at all. Choutka and colleagues have recently reviewed this topic, summarizing existing information for post-acute infection syndromes (PAISs) following at least 15 different pathogens, of which 12 are viruses and include SARS-CoV-2. The features of the various PAISs run the gamut of human organ system involvement and clinical presentations, with some similarities – e.g., fatigue, exertional intolerance, sleep disturbances, neurocognitive and neuropsychiatric symptoms, significant impairment of overall performance status – and many differences.

It is possible that PASC/long COVID stands out among PAISs because of a convergence of factors. It is very uncommon for hundreds of millions of people around the world to experience infection with the same viral pathogen in the span of just 3 years. This creates an explosion of the population of people at risk for incident PASC/long COVID, and this explosion of an at-risk population has occurred at a time when scientific information can be shared faster than ever in human history. In that same 3-year window, the entire global scientific and medical community has very appropriately turned its attention to SARS-CoV-2/COVID-19 research, in what one of the authors of this report has heard referred to as “the COVIDification of science.” As importantly, the people living with and suffering from PASC/long COVID have also shared information with unprecedented speed and scale, and this has been a critical element in recognizing that surviving acute COVID-19 is by no means the end of the story.

The context provided by PAISs other than PASC/long COVID is important. What is already known about the features, pathogenesis, and potential treatments for other PAISs may be very informative for accelerating understanding of PASC/long COVID. Conversely, what we are learning about PASC/long COVID may very well apply to other post-acute infectious syndromes and may help identify and deploy new treatments for the many people living with those syndromes. Finally, by advancing the understanding of what recovery in the post-acute infectious period looks like, we can hopefully identify strategies for preventing the long-term sequelae that at present are mysterious, poorly understood, frustrating, and debilitating for many, many people.

**Preclinical Models**

There is currently limited information regarding long-term animal studies representing PASC. This is likely due to limitations in available funding, shortage of Biosafety Level 3 space nationally, as well as challenge of developing safe methods to transfer animals from BSL-3 to the more common BL-2 environments. It is clear that many of the human symptoms observed in PASC have been observed in short-term studies that illustrate a subset of human PASC symptoms. Studies in mice, hamsters, ferrets and nonhuman primates have illustrated different subsets of these manifestations. Currently, there is a coordinated effort to perform long-term studies supported by NIH Office of Research Infrastructure Programs (ORIP) in three species of nonhuman primates within the National Primate Research Centers. These studies are being conducted at Tulane National Primate Research Center (African green monkeys), Emory National Primate Research Center and California National Primate Research Center (rhesus macaques), and Southwest National Primate Research Center (baboons). These studies have been harmonized in both study design...
as well as analyses, and will likely provide highly relevant animal models to study the pathogenesis and therapeutic interventions for PASC.

There are significant challenges regarding the study of animal models in PASC. Some models are quickly fatal and preclude longer terms study. Additional hurdles exist regarding the limitations in BSL3 space as well as the logistics, cost, and limited funding available to develop new models. Nevertheless, studies which are ongoing will likely provide new insights for the mechanism of PASC, novel therapeutic targets, and biomarkers to evaluate during clinical and animal studies.

A Call to Action
The Johns Hopkins University Coronavirus Resource Center estimates that there have been over 620 million cases of COVID-19 as of early October 2022. If even 1% of those people develop some form of PASC/long COVID, it would be millions of people worldwide who are in need of help – and that’s if the last ever SARS-CoV-2 infection in the world happened as these words are being typed. As with acute COVID-19, the magnitude and urgency of the need demand a response that surpasses traditional approaches of preclinical studies from which percolate over the course of years potential therapeutic targets that are then tested in multiple sequential translational and clinical studies.

Thankfully, the world has learned much from the response to the first 3 years of the global pandemic. We have learned that things can move much faster when siloes are taken down, and when basic scientists, translational scientists, clinical trialists, statisticians, informaticians, epidemiologists, clinicians, regulatory scientists, policymakers, and patients all get together and communicate frequently, clearly, and multidirectionally. We have learned that it is possible to test multiple agents simultaneously and in ways that respond to evolving knowledge while still allowing for robust conclusions to be drawn. We can hone our basic science investigations with feedback from the trials, designing trials that test mechanistic hypotheses at the same time as addressing the questions of safety and efficacy of an intervention. We can accelerate the deployment of therapeutically promising mechanistic findings by ensuring that clinical trial teams are up to date on the most current preclinical studies.

The NIH ACTIV public-private partnership is a testament to the power of these strategies. In just over 3 years, 800 potentially useful therapeutic agents have been assessed for prioritization into trials. Of those, 29 agents and combinations have completed testing in well-designed clinical trials and have been either more widely deployed or appropriately shelved as treatments for acute COVID-19, with several agents still under investigation or preparing to be reported. This was accomplished while the community was dealing with all of the other challenges presented by SARS-CoV-2 – in other words, these successes have been realized under extreme physical, mental, emotional, financial, and operational duress. If the lessons learned can be ported to PASC/long COVID, we have an opportunity not only to help the millions worldwide who are or will be living with the long-term sequelae that follow SARS-CoV-2 infection, we also likely have the opportunity to help the millions more worldwide who are living with the puzzling and frustrating long-term sequelae of other infections as well.
In February 2021, the NIH established the *Researching COVID to Enhance Recovery (RECOVER) initiative* to study the long-term effects of COVID, as well as why some people fully recover while others develop PASC. As of December 2022, RECOVER studies are ongoing.
Barriers to Improving Outcomes in PASC: What We Do Not Know
Michelle Gong, MD, MS, Albert Einstein College of Medicine – Delivered October 4, 2021

- Researchers and clinicians need not only to be able to differentiate PASC from similar post-acute illnesses, but also to predict the trajectories of patients with PASC.
- PASC differs greatly from other post-acute symptomologies in the persistence of symptoms in patients who are not hospitalized.

Research on COVID-19 has focused primarily on acute infection and mortality; however, there have been over 620 million SARS-CoV-2 infections worldwide (as of 10/20/2022), and a significant proportion of survivors are subject to long COVID/PASC. The many domains of PASC symptoms include pulmonary, fatigue, psychiatric, and neurocognitive symptoms, which may have varying persistence, association with severity of infection, predictiveness of outcome, and comparison to other post-acute illnesses from severe disease (e.g., acute respiratory distress syndrome [ARDS], sepsis) of SARS-CoV-2 infection worldwide, and a significant proportion are subject to long COVID/PASC. The many domains of PASC symptoms include pulmonary, fatigue, psychiatric, and neurocognitive symptoms, which may have varying persistence, association with severity of infection, predictiveness of outcome, and comparison to other post-acute illnesses from severe disease (e.g., acute respiratory distress syndrome [ARDS], sepsis).

The consequences of PASC can be immense and devastating. The National Heart, Lung, and Blood Institute (NHLBI)-supported Prevention and Early Treatment of Acute Lung Injury (PETAL) network developed a prospective observational cohort to track the natural history of COVID-19 and PASC. At one month post discharge from the hospital, 56% of patients reported new or worsening cardiopulmonary symptoms and 84% reported that they had not returned to pre-COVID-19 levels of functioning. Additionally, patients are subject to financial instability and hardships, with approximately 23% reporting that they had used all their savings due to their illness or hospitalization and approximately 20% reporting job loss or job change.\(^\text{11}\)

To better understand the effects of these sequelae, researchers need to determine the similarities and differences between post-acute COVID-19 and other post-acute illnesses. These comparisons have implications for the ability to better phenotype PASC to improve management of symptoms, the lives of affected individuals, and health disparities.

**Definition of PASC**
Researchers are hampered in determining the full impact of PASC due to the lack of a clear definition for the disorder. For example, the National Institute for Health and Care Excellence defines PASC as signs and symptoms that develop during or after an infection consistent with COVID-19 that continue for greater than 12 weeks and that are not explained by alternative diagnosis. However, the vast array of PASC symptoms makes it difficult for researchers to differentiate PASC symptoms from the symptoms of many other disorders with the same effects such as pneumonia or ARDS.
For example, pneumonia patients are known to have persistent, systemic symptoms, including many symptoms of PASC (e.g., cough, shortness of breath, confusion). On average, post-acute symptoms of pneumonia last for three weeks, with 25% of patients experiencing symptoms beyond 3 weeks. While the duration and frequency of symptoms are similar across older and younger patients, hospitalized patients had a longer duration of symptoms than non-hospitalized patients.

Comparatively, 25% of COVID-19 patients treated at Montefiore Medical Center (in- and outpatient populations) have reported persistent COVID-19 symptoms at 1-, 3-, and 6-month post infection. Of over 360 patients, 70-86% report persistent symptoms, and most symptoms are rated as moderate to severe, contributing to worse physical (84% of patients), cognitive (57% of patients), and emotional (68% of patients) states compared to pre-COVID-19 status. The most commonly reported symptoms include fatigue, breathlessness, pain, anxiety, and drowsiness; except for changes in taste, all categories of symptoms remain persistent even 6 months post infection. (Montefiore, located in the Bronx in New York City, was at the epicenter of the original COVID-19 outbreak and thus was faced with many COVID-19 patients with long-term symptoms.)

PASC Pulmonary Symptomology
While some researchers have tried to compare PASC post-intensive care outcomes to post-intensive care syndrome, pulmonary symptomology post-intensive care for COVID-19 patients may differ from other post-intensive care syndromes due to decreased use of formal intensive care unit (ICU) treatment for COVID-19 patients; critical COVID-19 patients may instead be offered high-flow nasal canula oxygen as an alternative treatment. At Montefiore, Dr. Marjam Islam, one of the founders of the CORE Clinic, received a grant to perform pulmonary ultrasounds on COVID-19 patients. To date, the ultrasounds have shown abnormal findings indicative of edema or interstitial changes, and scored scans correlate with desaturation when the patient completes a 6-minute walk. Most patients are capable of achieving only 52% of the predicted function for their age and status, indicating that COVID-19 persistent symptoms have significant pulmonary implications.

Other studies have shown that patients with post-COVID-19 ARDS symptomology have similar outcomes to those of non-COVID-19 ARDS patients, with severity of the original illness impacting long-term symptomology. For example, spirometry results show non-COVID-19 ARDS patients may improve for up to one year and then fail to progress further. COVID-19 ARDS patients show similar trends in function up to 12-months, but longer-term data are not yet available to determine whether the progression also
plateaus for these patients. The most affected parameters for both categories of patients are the diffusion capacity and 6-minute walk: diffusion capacity findings are similar between COVID-19 ARDS and non-COVID-19 ARDS, but the 6-minute walk results seem to be better for patients with COVID-19 ARDS.\textsuperscript{13,14}

Despite these similarities, PASC differs greatly from other post-acute symptomologies in the persistence of symptoms in patients who are not hospitalized. Unlike studies of patients with post-acute sepsis or acute respiratory failure, non-hospitalized COVID-19 patients have symptom burdens similar to hospitalized COVID-19 patients. The main difference between hospitalized and non-hospitalized PASC patients is the degree of disability, with hospitalized patients suffering from greater disability.\textsuperscript{13}

**PASC Neurological Symptomology**

Fatigue is a well-documented symptom of many viral syndromes (e.g., coxsackie, MERS, SARS-CoV), but most post-infectious syndromes that present as fatigue are poorly characterized and poorly diagnosed. Fatigue and weakness are also among the most common and persistent PASC symptoms, with 50–74\% of PASC patients reporting these symptoms at 12 months post-infection. Researchers have found similar findings in ARDS, with 66\% of patients reporting continuing fatigue at 12 months and 46\% reporting both fatigue and impaired physical function.\textsuperscript{15,16} However, unlike other post-acute illnesses, PASC fatigue may be worse in hospitalized patients and much worse in patients with severe COVID-19 infections.\textsuperscript{13}

PASC can also cause neuropsychiatric symptoms, particularly anxiety and depression. Over one-third of patients report anxiety and/or depression with acute illness, and 10–12\% of patients report developing new anxiety and/or depression 3–6 months post-infection. These psychiatric conditions seem to worsen over time, such that patients who did not have severe infection report increased anxiety and depression 12 months post-infection. These reported PASC-related neuropsychiatric symptoms are similar to those observed in patients with ARDS 1-year and 2-year post illness, with non-severe patients further out from infection reporting increased depression.\textsuperscript{17} However, PASC-related neurological findings are confounded by the increased prevalence and incidence of anxiety and depression among non-COVID-19 patients during the pandemic.\textsuperscript{18} In fact, a random sampling of the US population
found more than a doubling of Americans reporting moderate to severe depressive symptoms, as well as increased generalized and social anxiety, during the pandemic compared to before the pandemic. This makes it difficult to determine whether these neuropsychiatric symptoms are from the consequence of the pandemic or the acute SARS-CoV-2 infection.

Predictors of depression and anxiety post-COVID-19 infection and post-ARDS are similar and include ratio of arterial oxygen partial pressure to fractional inspired oxygen (PaO2/FiO2), mechanical ventilation duration, older age, and female sex. The greatest stressors related to anxiety and depression for PASC patients are social and economic stressors related to COVID-19 infection itself—including feeling alone, difficulty with family and relationships, and difficulty with finances. These factors may not improve immediately following COVID-19 infection and therefore it makes sense that these symptoms may increase over time before eventually resolving.

PASC also has a prolonged, persistent impact on neurocognitive functioning that appears similar in kind and duration compared to the impact of other post-acute illnesses, such as ARDS and sepsis. Approximately 12% of COVID patients presenting with acute neurological symptoms require imaging, most often (44%) due to an unexplained altered mental state. Patients may suffer from impaired concentration or impaired memory, with nearly 20% of COVID-19 patients reporting persistent problems 3-6 months post-infection. In the Montefiore CORE Clinic, 41% of patients exhibit significant cognitive impairment on the Montreal Cognitive Assessment (MOCA) blind test. Patients with ARDS and sepsis who have prolonged, persistent neurocognitive deficits generally improve during the first-year post-acute infection but then plateau, and longitudinal studies show that the symptoms are long-lasting and often associated with job loss. COVID-19 patient data have begun to show similar trends in persistence and recovery.

However, PASC patients again have worse impairment among those with more severe disease during acute infection. In fact, the most extreme deficits observed among ventilated patients shows a greater deficit than the average expected 10-year decline for patients between the ages of 20-70. Many studies have not accounted for baseline cognition, however. In similar studies in sepsis and ARDS, predictors of cognitive dysfunction include not only disease factors—such as severity of illness during onset (i.e., hypoxia, hypotension) and injury during treatment (i.e., excessive sedation, delirium)—but also older age and pre-existing decline. This is important because many of the most affected COVID-19 patients are either elderly or affected by comorbidities. Longitudinal studies of COVID-19 patients are thus needed to observe pre-infection and post-infection neurocognition to best understand the full impact of the neurocognitive decline in these patients. While some studies have begun, they are in limited patient populations.
Phenotyping PASC

Researchers and clinicians need not only to be able to differentiate PASC from similar post-acute illnesses, but also to predict the trajectories of patients with PASC. Severe COVID-19 infections lead to a spectrum of different severities of symptoms and impairments, ranging from no effect to long-lasting, severe impacts. However, knowledge is currently lacking regarding predictors of higher likelihood of different symptoms and symptom categories, in part because PASC symptoms overlap with post-acute symptoms of ARDS and, to a lesser extent, with myalgic encephalomyelitis/chronic fatigue syndrome. As a result, all symptoms and findings are lumped together, blurring distinctions between phenotypes with different trajectories of recovery, impact on function, mechanisms of injury, and potential treatments.

Differentiating these phenotypes is important because treatments may be available for some of PASC’s underlying causes. For example, effective interventions aimed at limiting acute and iatrogenic injury include lung protective ventilation, early mobilization, decreasing delirium, minimizing unnecessary sedation, and avoiding benzodiazepines. Additionally, effective interventions aimed at promoting functional recovery or compensation include physical and occupational therapy and cognitive therapy. However, it is less clear whether there is a need to treat persistent injury or dysregulation after acute COVID-19 infections as such continuing pathology have not been clearly defined.

Research is also needed to determine whether treatment for acute COVID-19 (e.g., oxygen, corticosteroids, monoclonal antibodies, remdesivir) also modulate PASC. For example, certain myths of treatment, such as “happy hypoxia,” (i.e., patients presenting with profound hypoxemia yet without proportional signs of respiratory distress) led to insufficient treatment, which may have long-lasting health implications. Additionally, acute treatment with corticosteroids, frequently used to treat acute COVID-19, was significantly linked to continued muscle weakness and fatigue even up to one-year post-illness.13

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PASC and COVID-19 Variants
COVID-19 has evolved from the start of the pandemic in early 2020 to more recent variants, such as Delta. Data are lacking to compare long-term outcomes of individuals infected by COVID-19 early in the pandemic versus those infected by more recently identified variants, in part because later patients do not have enough follow-up for a robust comparison. However, there are some suggestions that PASC will evolve with the new variants. For example, the Delta variant is affecting younger patients with more severe acute illness, and this change in primary patient population may in turn affect the presentation of PASC. Researchers may need to rely less on cross-sectional surveys and focus more on collecting long-term analyses of patients in order to better understand the differences in both symptoms and recovery between PASC from early and later COVID-19 variants.

Gender and Racial Disparities in COVID-19
Gender and racial disparities have been reported in acute COVID-19 infections, and recent data suggest that they may be present among PASC patients as well. According to multiple reports, PASC disproportionately affects females, although reports on the impact of PASC for minorities have produced mixed data. No studies have investigated disparities in accessing post-COVID-19 care, but such disparities are likely given evidence of racial and social-economic disparities in access to and quality of post-acute care for other illnesses. For example, black patients and patients with Medicaid are less likely to be transferred to long-term acute hospitals after intensive care unit stays. Additionally, minorities report less improvement in activities of daily living after discharge from hospitalization. Further research is needed to identify and address racial and gender disparities for COVID-19 and PASC treatment.11,13,26–30
Probing Disease Mechanisms of Long COVID-19

Akiko Iwasaki, Yale University School of Medicine – Delivered October 28, 2021

- PASC is a new syndrome in a history of post-acute infection syndromes that detrimentally impair patients after viral and non-viral infections.
- The symptomologies of PASC are heterogenous, which may suggest heterogeneous pathologies and etiologies.
- Researchers need to understand the immunological and physiological determinants of PASC pathogenesis to inform rational diagnosis and therapies.

History of Post-Acute Infection Syndromes

Prior to the discovery of PASC, researchers were aware of numerous other post-acute infection syndromes caused by both viral and non-viral infections (i.e., bacterial or parasitic pathogens). In a recent review, Drs. Iwasaki, Viraj Jansari, Mady Hornig, and Jan Choutka identified numerous infections and their known post-acute infection syndromes (PAIS) (e.g., Ebola and post-Ebola syndrome, Polio and post-polio syndrome).9

While PAIS have distinct symptomologies, they share some main symptom categories, including (1) exertion intolerance and fatigue; (2) flu-like sickness and “sickness behavior” (e.g., malaise, muscle pain); (3) neurological and/or neurocognitive deficits (e.g., impaired concentration or memory); (4) rheumatologic pain (e.g., chronic or recurrent joint pain); and (5) trigger-specific symptoms (e.g., eye problems post-Ebola). Despite well-documented cases of PAIS, they have been under researched, and none have received as much attention as PASC.

Heterogeneity of PASC

Consistent with the characteristics of PAIS, PASC patients experience a myriad of symptoms weeks and months after infection—most commonly fatigue (78% of patients), post-exertional malaise (72% of patients), and cognitive dysfunction (55% of patients).31 The majority of PASC patients also have evidence of multiple organ involvement during both acute and post-acute infection. Overall, PASC is associated with hundreds of symptoms involving numerous organ systems, including neuropsychiatric; head, eyes, ears, nose, and throat; endocrine; cardiovascular; pulmonary; musculoskeletal; gastrointestinal; and systemic symptoms. Researchers must assess whether heterogeneous causal pathways underlie these diverse symptoms and whether clustering patients by symptoms could therefore help elucidate PASC pathogenesis.

PASC symptoms also have a heterogeneous timeline. In general, symptoms that start off severe or very severe may decline and be replaced by mild to moderate symptoms, but the recovery curve flattens, suggesting that patients may go on to suffer for months and potentially years after the acute infection. Within this overall timeline, however, studies have shown distinct patterns. One study has suggested that patients with fewer symptoms (less than 13) were more likely to recover within 90 days of their primary infection while patients with 13 or more symptoms had not recovered even as far out as month 7 post-acute infection.31 This finding suggests that the involvement of multiple organ systems during acute infection may dictate the extent and longevity of PASC. This study also tracked symptoms by organ class.
over time and showed that most patients continue to report fatigue at 7 months after onset, whereas other symptoms such as fever resolve over time. The study also showed that numerous reproductive and endocrine symptoms were reported, particularly linked to menstruation. Other studies reported immunologic and autoimmune symptoms, suggesting that autoimmune disease may be occurring in PASC.

### Categories of PASC
A significant portion of patients infected with COVID-19 develop PASC, although studies have to date disagreed on the precise proportion of the population affected. In part, this statistical disagreement is due to the varied definitions in place across different systems nationally and internationally. Another key driver to the statistical uncertainty is the heterogeneity of PASC symptomology. Based on this heterogeneity, researchers have begun work to differentiate potential PASC pathophysiologies. For example, among those patients with severe, hospitalized cases of COVID-19, approximately 50% of patients have long-term symptoms—primarily fibrosis, tissue damage, and organ damage. Of patients who had mild or potentially including asymptomatic cases of COVID-19 that did not lead to hospitalization, approximately 5 to 35% develop long-term symptoms—primarily fatigue, brain fog, post-exertional malaise, and dysautonomia. These two complexes are increasingly referred to as Post-Severe COVID-19 Syndrome (PSCS) and Post COVID-19 Fatigue Syndrome (PCFS), respectively.

Along with different symptomologies, PSCS and PCFS also have different patient demographics. One systematic review revealed that 54% of COVID-19 survivors who were hospitalized develop PCSC. The patients from that review were primarily male (56%) and hospitalized (79%) and had an average age of 54.4 years. However, another study showed that middle aged women are more likely to develop debilitating PASC symptoms, and a third study claimed that compared to males from the same age group, women under 50 years old are 5 times more likely to report not recovering from symptoms, 7 times more likely to become more breathless, and more likely to have greater disability.

For patients with PCFS, one study of non-hospitalized patients found that lower baseline levels of SARS-CoV-2 IgG, anosmia, and diarrhea during acute COVID-19 infection were associated with higher risk of
developing long-term symptoms. The same study found that male sex was associated with lower risk for long-term symptoms. Having lower baseline IgG may mean that a failure to clear the primary infection may be responsible for the development of PCFS.

Possible Causes of PASC
Numerous hypotheses have been offered about the cause of PASC and further research is needed to assess them. While some hypotheses have focused on tissue damage during acute infection, dysbiosis, and latent viral reactivity, two major hypotheses include:

1. The establishment of viral reservoir in various tissues throughout the body could cause chronic inflammation, leading to PASC symptomologies and even triggering organ-specific dysfunction where the viral reservoirs remain.
2. Acute COVID-19 infection triggers autoimmune responses—whether in T-cells, B-cells, or both—that target organ or cell tissues that then trigger long-term consequences and drive reported symptomologies.

Early evidence has been found to support these two major hypotheses. Regarding viral reservoirs, intestinal biopsies of patients taken 92 days after symptom onset showed that five out of 14 patients had positive staining for viral nucleic capsid and three out of 14 patients were PCR positive for viral RNA. Another study of biopsies and surgical specimens from five patients who recovered from COVID-19 (and who had negative nasopharyngeal swabs) revealed the presence of viral protein and RNA in multiple tissues, including the gastrointestinal (GI) tract and lymph nodes. These findings indicate the possibility of a viral reservoir or “viral ghost” persisting in tissues after acute infection.

The second hypothesis is supported by the demonstration of a set of autoantibodies that develops during acute COVID-19 infection. Based on these findings, Drs. Iwasaki and Ring’s team applied the Rapid Extracellular Antigen Profiling (REAP) technology that comprehensively and genetically barcodes a library of ~3,000 extracellular and secreted proteins (exoproteome) displayed on yeast. REAP enabled the researchers to investigate IgG from plasma and serum from COVID-19 patients and to decode the antigens expressed. Researchers found a number of autoantibodies and immune factors that are targeted by
autoantibodies that appear to deplete the subsets of cells required to combat viral infection. These findings demonstrate the development of autoantibodies that could have a specific pathogenic effect in COVID-19 patients. The research team is following patients over one year post-study to determine the long-term consequences of these autoantibodies and determine whether autoantibodies persist. Numerous other researchers have reported demonstrating autoantibodies in acute and long-term COVID-19 infection.

Other researchers, based on alternative hypotheses, have investigated the inflammatory and immune signatures of PASC. One study followed patients who recovered from COVID-19 who either did or did not have PASC. The researchers examined early and late cytokine counts and found that elevated IL-6 was maintained in PASC patients, even at the late timepoint, whereas other cytokines were not significantly different at the acute or late stage. Additionally, there was not a large difference in SARS-CoV-2 IgG levels in PASC versus non-PASC patients. These finding is interesting because in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS)—that happen with certain PAIS—researchers have reported much longer timelines for resolution of acute levels of IL-1α and TNF and increased long-term IL-6 levels. Continued monitoring of PASC patients is required to determine whether the current findings continue to hold for as long as they do in ME/CFS patients. Although these findings suggest that circulating cytokines may be a biomarker for PASC, the same research team found few differences in any T-cell parameters, indicating that there are no overt differences in T-cell responses between patients who do or do not develop PASC after an acute COVID-19 infection.
Another study demonstrated that PASC is characterized by immune cell subsets. For example, researchers observed a time-dependent increase in the number of T follicular helper cells—necessary for clearing the virus—against the nuclear capsid fighting COVID-19 infection, whereas in the healthy capsid the number declines over time. Additionally, PASC patients persistently experience elevated levels of anti-N-IgG compared to non-PASC patients. These findings suggest persistence of nucleoprotein in PASC. The same study demonstrated reduced CD8 memory subsets against viral proteins in PASC patients versus non-PASC patients. Together these findings may suggest persistent viral antigens drive the development of antibody responses while reducing the memory of antibody cells in PASC patients.

**COVID-19 Vaccine and PASC**

Despite the heterogeneity of symptoms and the potential heterogeneity of the pathophysiology of PASC, one treatment has shown some potential for improving PASC symptoms. As tracked by the LongCovidSOS group, vaccination for COVID-19 improved PASC symptoms in roughly 60% of patients. However, about 19% of patients instead reported worsening symptomology after vaccination. These findings illustrate that regardless of vaccine type, PASC symptoms are modulated by COVID-19 vaccination. When analyzed by types of symptoms, the Moderna vaccine had slightly better improvement rates for
fever, muscle pain, and sore arm, but most symptoms were relatively similar in improvement rates regardless of vaccine received.

Another pre-print publication posited that vaccination seems to double remission rates of PASC symptoms. The Preprint showed the results of a simulated targeted trial of vaccination in PASC patients. The heterogeneous reaction to the COVID-19 vaccination may be explained by the two major hypothesized causes of PASC:

1. If viral reservoirs are causing PASC, COVID-19 vaccination may improve symptoms by eliciting robust antiviral antibodies to clear the viral reservoir.
2. If an autoimmune response causes PASC, COVID-19 vaccination may divert autoimmune lymphocytes through innate and adaptive cytokines.

Ongoing Studies
Research is ongoing to understand the immunological and physiological determinants of PASC pathogenesis and to inform rational diagnosis and therapies. Researchers at Yale, Mount Sinai School of Medicine, and Howard Hughes Medical Institute have developed three cohorts of COVID-19 patients, all with suspected PCFS:

- Cohort A: Cross sectional Long COVID cohort
- Cohort B: Longitudinal Long COVID vaccine cohort
- Cohort C: Longitudinal symptom-based Long COVID cohort

Each cohort will investigate a different aspect of PASC pathogenesis. The study design will collect saliva, blood, and symptomologies from PASC patients pre-vaccination, 6 weeks post first vaccine shot, and 12 weeks post first shot. A separate cohort, leveraging the Long IMPACT Yale study, is capturing symptomologies and immune responses in PSCS patients to understand changes beginning at hospital.
discharge. The study will compare acute phase and post-acute phase parameters to characterize the full extent of physiological changes associated with the transition between COVID-19 acute infection and long-term COVID-19 symptoms.

Future Research

**Drivers of Variability**
Researchers do not yet understand why variability has been observed in PASC patients’ responses to COVID-19 vaccines, vaccines for other infections, and secondary infections. If an autoimmune response explains PASC, then patients would be expected to experience a flare in symptoms from the increased inflammation associated with secondary infection or vaccinations. If PASC is caused by autoreactive cells, then inflammation leading to release of the same type of cytokine would potentially cause a flare in symptoms. The heterogeneity of the disease is complicating researchers’ ability to discern the reasons for the variability of reactions by PASC patients. Sufficient number of patients are required to draw better conclusions.

**Intersection of Clotting and Immune Systems in PASC**
Some researchers have noted the presence of thromboses or microclots in PASC patients. The clotting system, which is activated by virus infection, cytokine signaling as well as immune cell activation, works to block infection by confining pathogens or repairing tissue damage. Therefore, chronic inflammation could predispose individuals to clotting. However, additional research is needed to fully understand the underlying mechanisms of the relationship between the immune system and clotting factors as they relate to COVID-19 infection and PASC.

**PASC versus PAIS**
Physicians may be challenged to identify whether COVID-19 patients, particularly those with severe cases requiring hospitalization, have developed PASC or another PAIS, or even infection-unrelated post-ICU syndrome. Different post-acute infection syndromes have different symptomologies, which are often the best way to differentiate between syndromes. For example, PASC is associated with chronic respiratory failure, cardiac arrythmia, hypercoagulability, encephalopathy, peripheral neuropathy, amnesia (memory difficulty), diabetes, liver test abnormalities, myocarditis, anxiety, and fatigue, none of which are common symptoms for any other PAIS. One reason that disentangling PAIS pathophysiology and symptomologies is difficult is that researchers have not invested heavily into researching any other PAIS aside from PASC. PASC has revolutionized the amount of attention paid to an individual PAIS and may pave the way for better research on other forms of PAIS in the future.
Neurological Symptoms During and After COVID-19: What Can We Learn from the CSF?
Shelli Farhadian, MD, PhD (Yale School of Medicine) - Delivered November 4, 2021

AUTHOR’S NOTE: In September 2022, a retrospective cohort study of older adults published in Journal of Alzheimer’s Disease found that people aged 65 or older were at significantly increased risk for new diagnosis of Alzheimer’s within 360 days after the initial COVID-19 diagnosis.

- Patients with acute COVID-19 infection and PASC have presented with varied neuropsychiatric symptomologies.
- Better studies are needed to assist in differentiating the onset of new neuropsychiatric symptoms due to COVID-19 infection as opposed to worsening of pre-existing conditions.
- Neuropsychiatric symptomologies in COVID-19 and PASC may be related to autoimmune reactions in the central nervous system.
- Preliminary research in cerebrospinal fluid has suggested that the central nervous system may have an autoimmune reaction separate from the blood.
- Further research is needed to determine whether this central nervous system reaction is related to neuropsychiatric symptoms in COVID-19 infection and PASC.

Neurocognitive Symptoms in COVID-19 Patients
Since the start of the epidemic, although SARS-CoV-2 is clearly a respiratory virus, patients have suffered from a myriad of symptoms. Particularly interesting among these symptoms are neurological effects, especially headache and dizziness, and less frequently seizure and stroke. Early reports from China and Chile suggested that around one-third of COVID-19 patients experienced neurological symptoms and more recent studies show that 13% of patients with COVID-19 presented with a new neurological diagnosis. Most of these new diagnoses were non-specific (e.g., toxic/metabolic encephalopathy). Even more recently, a study at Yale found that COVID-19 patients in Connecticut who required a neurological consultation were primarily White (48%) even though Black (25%) and Hispanic (25%) patients were disproportionately represented among COVID-19 patients overall, compared to Connecticut’s demographic make-up (12% and 17% respectively).

Potential Pathways of Neurologic Impact
While viruses can invade the brain directly, numerous other pathways by which respiratory viruses can cause neurological symptoms have been proposed, including systemic inflammation, vessel thrombosis, endothelial damage, and autoimmunity. While SARS-CoV-2 has the potential to infect neurons, viral neuro-invasion by SARS-CoV-2 is rare. In the study finding 13% of COVID-19 patients had neurological symptoms, no patients were diagnosed with encephalitis or meningitis, meaning that the patients did not experience swelling in the brain or brain stem, and the patient’s cerebrospinal fluid (CSF) did not indicate overt signs of inflammation. One of the largest autopsy studies of COVID-19 patients showed that of 43 brains examined, 53% were positive for SARS-CoV-2 RNA or protein, but the viral presence was not associated with neuropathological changes. Another study of 13 patients showed widespread microvascular injury without any detectable virus. Studies have shown that overall neurons do not appear to be infected but the vascular epithelium appears to be prone to infection and inflammation.
Researching Central Nervous System Immune Response to COVID-19

Although autopsy studies suggest that neuronal invasion is rare or short-lived, such studies are limited and cannot determine what is happening in the central nervous system (CNS) in living adults. Other studies have shown evidence of CNS immune activation in affected patients, including microglial nodules, activated astrocytes, cytotoxic t-cell infiltration of the brainstem and meninges, and microvascular injury. To further investigate this evidence, researchers have turned their focus to researching CSF—a known window to the brain. CSF includes cells that have crossed the blood-brain barrier, as well as proteins that are produced within the brain. The IMPACT Yale Biorepository collected samples from over 350 hospitalized patients with acute COVID-19 including blood, nasopharyngeal swabs, saliva, urine, and, in some cases, CSF to allow researchers to perform immune cell profiling and examine the CSF supernatant to observe antibodies, soluble cytokines, and markers of blood-brain barrier disruption.

Previous research suggested that cytokine storm or other cytokine responses in the peripheral blood was associated with severe COVID-19 infection. To test this theory, the Farhadian group, led by Dr. Eric Song, investigated measured levels of inflammatory cytokines in the blood and CSF in COVID-19 infected patients from the IMPACT cohort and healthy controls. Dr. Song found that the cytokines expressed in the blood differed from those expressed in the CSF. To confirm these findings, the Farhadian group performed single cell RNAseq and found that different pathways were upregulated in the blood and CSF in COVID-19 patients but not healthy controls. These findings suggest a compartmentalized response to COVID-19 in the CNS.

Antiviral and Autoimmune Response

To better understand the response of antiviral antibodies in the CNS, Dr. Michael Wilson at the University of California, San Francisco used his recently developed multiplex ELISA test to look for antibodies that react against SARS-CoV-2 epitopes (e.g., spike, RBD, nucleocapsid) in the same IMPACT participants. Dr. Wilson found different antibody profiles in the CSF and plasma of 5 patients tested. All patients had anti-SARS-CoV-2 antibodies in both the blood and serum, but the CSF antibodies targeted different epitopes than those in the blood. These findings suggest that antibodies in the CSF may be reacting to local CNS COVID-19 infection.
To better understand the nature of the CSF-specific antibodies, researchers investigated clonally expanded B-cells. They found that patients with COVID-19 had an increased frequency of CSF B-cells, and a single cell RNA sequencing of immunoglobulin genes revealed expanded B-cell clones in PBMC and CSF. Researchers used these clonal B-cell sequences to create patient-derived monoclonal antibodies (mAbs) and tested these mAbs (five from the CSF and four from the blood) for reactivity against spike protein. They found that one mAb in the CSF and two in the blood were reactive against COVID-19. They then tested whether the mAbs were autoreactive by performing mouse brain immunostaining; three of the five CSF derived mAbs were reactive against mouse brain tissue, including the single mAb that was reactive against the spike epitope, suggesting cross reactivity between antiviral antibodies and brain tissue. Further research has shown that anti-neural autoantibodies are common (appearing in five of seven tested cases) in the CSF of COVID-19 patients with neurological symptoms. This finding demonstrates a high burden of autoimmunity in the CNS of acute COVID-19 patients. Further research in this area is needed because only some of the autoimmune antibodies have been validated through cell-based assays and further data may be leveraged from staining studies of post-mortem human brains. While deeper, isolated B-cell testing would be interesting, the total number of B-cells in COVID-19 patients CSF is limited and not conducive to isolation experiments.

In a single case study of a patient with COVID-19 related psychosis further supports proposed autoimmunity in the CNS. A 30-year-old male patient with no previous neurological diagnosis presented with fever and malaise upon development of COVID-19. Early development of delusional symptoms eventually worsened and by Day 22 the patient was suffering severe paranoid delusions. After being admitted to Yale New Haven Hospital, the patient was treated with intravenous immunoglobulin, a treatment often used against autoimmune responses, and mounted a full recovery. The patient agreed to
donate blood and CSF during treatment and researchers confirmed that the patient’s CSF showed evidence of autoimmunity with reactivity against brain tissue.

Based on all the above findings, researchers concluded that although SARS-CoV-2 is rarely detected in the CSF, anti-viral antibodies are present in the CSF and differ from those in the blood. The CSF immune cells show divergent activation pathways when compared to peripheral blood immune cells in acute COVID-19 patients. Most neurologically symptomatic COVID-19 patients tested had autoimmune antibodies in their CSF, although the clinical significance of the presence of these antibodies is unknown.

Central Nervous System Immunity and PASC

Addressing Confounding Factors
The epidemiology of neurological symptoms of PASC are poorly understood, in large part because of the poor quality of studies capturing data on PASC patients. Many of these studies are observational and do not require a confirmed diagnosis of COVID-19. Additionally, studies rarely separate new onset neuropsychiatric disease from exacerbation of existing diagnoses. One of the most widely cited studies was an online survey of mostly White (85%), female (78%) patients, of whom only 27% had a PCR or antibody test diagnostic of COVID-19. Another widely cited study correlated post-COVID-19 PTSD with a history of psychiatric disorders, highlighting the need for better understanding of prior comorbidities. Another confounding factor in understanding PTSD, in particular, among PASC patients is that the COVID-19 pandemic was a traumatic experience for everyone, with between 10% and 55% of healthcare workers reporting PTSD in studies regardless of COVID-19 diagnosis. Work from the previous SARS outbreak showed that individuals who were isolated, worked in high-risk settings, or had a close relation affected by the disease were two to three times more likely to have PTSD. Furthermore, 25 to 35% of ICU survivors reported PTSD, even in non-COVID-19 related cases.
One study that accounts for some of these factors when reporting on the epidemiology of new onset neurological and psychiatric outcomes in PASC patients was published by Dr. Luciano Taquet. The study compares individuals diagnosed with COVID-19 to individuals diagnosed with the flu or other respiratory tract infections during the same time period. In the study, the incidence of any neuropsychiatric outcome was 33% and the incidence of first neuropsychiatric outcome was 12%. The study found that PASC is associated with a wide range of neurological and psychiatric diagnoses, although the effect is dampened in patients who never had encephalopathy.\(^6\)

Other researchers leveraged the UK Brain Bank Study to investigate changes in the brain post-COVID-19 infection. 394 COVID-19 positive individuals who had pre-pandemic brain scans returned for a brain scan post-acute infection and were compared to 388 matched controls. Most COVID-19 patients had experienced mild or no symptoms but were included on the basis of an antibody test. Researchers found small losses of grey matter in brain regions, mostly those related to smell. The loss of grey matter was worse in the 15 patients who had been hospitalized for severe COVID-19. However, researchers are unsure of why these changes in the brain are occurring and have not yet established a causal link between COVID-19 and these brain changes.

**COVID-19 Mind Study at Yale**

Based on these findings, Drs. Serena Spudich and Shelli Farhadian undertook the COVID-19 Mind Study at Yale, an observational study of post-acute COVID-19 neurological symptoms. The study mirrors the Yale approach to researching acute COVID-19 patients, including collecting and testing blood and CSF; collecting a detailed history to understand acute and post-acute symptoms; and performing a battery of neuropsychiatric tests. The study is ongoing, but preliminary data in the first 27 participants—most of
whom are affected by cognitive impairment—show no major differences in blood c-reactive protein, blood CD4/CD8 ratio, CSF neopterin, CSF protein, or CSF/serum albumin ratio between PASC patients and normal controls. Researchers did identify a slight elevation in blood d-dimer levels in PASC patients compared to controls. While the CSF white blood cell count was not significantly elevated in PASC patients, the patients did have a slightly lower proportion of lymphocytes in CSF. The research team concluded that there are so far no overt immunological differences in the blood and CSF between PASC and control cases.

The researchers also investigated whether the anti-SARS-CoV-2 antibodies in COVID-19 patients’ CSF persist after acute infection. Preliminary findings show that most participants did have continued detectable antibodies against CSF full spike, CSF nucleocapsid, serum full spike, and serum nucleocapsid even at 300 days after acute infection resolution. Two participants in the study had received a COVID-19 vaccine, and both showed the lowest antibody responses. However, these findings seem to be particular to the participants and are not thought to be characteristic of what can be expected among the majority of individuals vaccinated for COVID-19.

Next steps for this study include continuing to (1) collect standardized neuropsychiatric testing to complement the symptom report, (2) assess CSF and blood immune markers with deeper immunophenotyping approaches, (3) perform antibody screening for the presence of autoantibodies in the CSF, and (4) capture neuroimaging to look for MRI correlates of PASC neurological symptoms. Future research should focus on further elaborating the difference between CNS and other systemic reactions, both in COVID-19 and other infectious diseases. Future research should also consider whether autoimmunity is driving the B-cell changes over time, particularly due to the cross-reactivity observed in antigens.
Targetable Plasma Correlates of COVID-19 Severity: PASC Relevance?

Mohamed Abdel-Mohsen, PhD (Wistar Institute) - Delivered October 18, 2021

- Due to inflammation reactions caused by cytokine storms, COVID-19 causes gut permeability, which in turn exacerbates systemic inflammation.
- Researchers have identified biomarkers of interest for microbial translocation and for inflammation, as well as more complex signatures from metabolomic, lipidomic, and glycomic analyses in COVID-19 patient samples.
- Future research is needed to determine whether these biomarkers also play a role in PASC.

Plasma Correlates and Infectious Diseases

Before the COVID-19 pandemic, evidence had already emerged of a correlation between reactions in the lung and gut microbiomes following acute infectious respiratory diseases. Lung infection or damage causes a systemic inflammatory response that can include a cytokine storm, or a dramatic increase in the small proteins responsible for controlling the activity of the immune system and blood cells. Two cytokines in particular, TNF-alpha and interferon-gamma, can injure the gut and increase intestinal permeability—the ability of naturally occurring bacteria and fungi to leave the gut and enter the bloodstream, spreading throughout the body. These bacteria and fungi can further increase inflammation and cytokine storms, creating a negative feedback loop of worsening lung damage and worse outcomes of infectious diseases.

These findings led researchers to try to identify a potential link between lung infection, inflammation, and gut damage from COVID-19. Dr. Mohamed Abdel-
Mohsen and his team analyzed samples from COVID-19 negative and COVID-19 positive patients to identify targetable gut-related correlates of COVID-19 severity in patients 50- to 65-years old (with equal numbers of male and female participants). Plasma samples were processed to analyze microbial translocation markers, inflammation markers, metabolomics, lipidomics, and glycomics. The first four measures were chosen for their known impact on acute lung infections, while glycomics were added for their known association with long-term impacts of chronic infectious disease (i.e., HIV).

Translocation and Inflammation Markers and COVID-19

One biomarker researchers examined was zonulin, which is both a marker and driver of tight junction gut permeability. Elevated zonulin levels caused by gut dysbiosis (changes in the composition of the gut microbiome) allow microbes to translocate outside of the gut, increasing cytokine storms, inflammation, and T-cell activation. Pre-pandemic research had thus already begun preclinical and clinical trials of zonulin antagonists for the treatment of infections associated with inflammation.

Researchers found that patients with moderate to severe COVID-19 had significantly higher levels of zonulin than those with mild COVID-19 or COVID-19 negative controls. Furthermore, those patients who died from COVID-19 had higher levels of zonulin than those who survived. These results suggest that zonulin, as a physiological driver of worsening COVID-19 symptoms, may be a target for future COVID-19 treatments.

To confirm that elevated zonulin was accompanied by bacterial and fungal translocation, researchers also measured levels of lipopolysaccharide (LPS)-binding protein, which indicate bacterial translocation, and beta-glucan, which indicates fungal translocation. Levels of both markers were increased in patients with moderate to severe COVID-19, with levels of bacterial translocation more elevated compared to controls than levels of fungal translocation. Because the escape of bacteria and fungi from the gut in turn cause an increase in neutrophils—a type of white blood cell—researchers also confirmed that moderately to severely ill COVID-19 patients had increased soluble CD14 and MPO levels, both of which are markers of neutrophil inflammation. These findings were further backed by studies of systemic inflammation and immune activation, which were significantly elevated among patients with moderate to severe cases of COVID-19.
These results are reflected in the broader COVID-19 literature, which has recently seen an uptick in publications linking the gut microbiome, changes in the microbiome, and COVID-19 severity. Researchers have also noted that the inflamed and leaky gut is more likely to occur in patients that are older, obese, or chronically ill; an infected person with a strong immune system and a strong gut is less likely to develop a leaky gut and therefore less likely to be subject to severe COVID-19 infection.

**Metabolomics and COVID-19**
Severe COVID-19 is also associated with a plasma metabolomic profile that reflects disrupted gut function. Metabolomic pathways known to be associated with gut dysregulation were found to be upregulated and downregulated in moderate and severe COVID-19 patients. Some of these pathways may prove to be useful targets for treatment of COVID-19 symptoms.
The first metabolomic biomarker is the amino acid citrulline, a known marker of intestinal function and absorption in the clinical setting. As systemic citrulline levels decline, patients’ intestinal function is reduced. Citrulline has also been shown in animal models to be a potential regulator of gut permeability.

Another potential metabolomic target for COVID-19 is tryptophan catabolism, which along with its associated metabolite is associated with dysbiosis in people living with HIV (PLWH), including those with successful viral suppression treatment, as well as with long-term complications of HIV. Tryptophan catabolism induction is associated with moderate and severe cases of COVID-19 and may explain complications of both acute and post-acute complications, such as PASC.

**Future Research**

The tryptophan catabolism pathway is the de novo pathway for the creation of nicotinamide adenine dinucleotide (NAD)—an organic molecule that binds to the active sites of certain enzymes and is a critical element of metabolism. Researchers may investigate the NAD pathway and metabolism pathways for other drivers of metabolic dysfunction that are caused by severe COVID-19 infection.

**Gut Biomarkers and PASC: An Area for Future Research**

Dr. Abdel-Mohsen’s findings raise a range of questions about PASC. Some questions that researchers are currently focused on answering include:

1. Does disruption of intestinal barrier integrity persist after convalescence and contribute to PASC?
2. Can modulators of tight junction permeability lessen COVID-19 severity and/or PASC?
3. Does SARS-CoV-2 infection directly and/or indirectly, through an associated cytokine storm, disrupt intestinal barrier integrity? What is the impact of established gut disruptors (e.g., alcohol abuse) on PASC?
4. Can some of the markers identified thus far predict the risk of disease progression and/or PASC if measured before or immediately after diagnosis?

Preliminary findings regarding the first question have revealed that PASC is associated with an increase in fungal translocation to the plasma and a lesser trend toward increased bacterial translocation. Researchers have two hypotheses to support these preliminary findings: (1) fungal translocation has been associated with long-term complications of another infectious disease (HIV) and/or (2) fungal translocation is a driver of chronic inflammation.

Increased fungal translocation may also be a reflection of the health, metabolic, and/or socioeconomic status of individuals prone to PASC. People who are older, with pre-existing metabolic syndromes, and/or with lower socioeconomic status are independently more prone to both fungal translocation and PASC. Researchers need to determine whether the identified correlation has shared causation.

Research has recently begun to compare PASC patients who are living with HIV and those who are HIV-negative to determine whether PLWH, particularly those with leaky gut, have an increased likelihood of developing PASC and whether that risk is related to markers of gut translocation. Patients treated with complement inhibitors for HIV are another population of interest for both acute COVID-19 and PASC studies.
Future treatments for consideration, if they have not already been tested, include antifungal medications, neomycin or other non-absorbable antibiotics, and/or fecal transplant. However, some antibiotics can actually increase the permeability of the gut. In fact, researchers may wish to determine whether current antibiotic or steroidal treatments used for acute COVID-19 infections are exacerbating systemic inflammation by exacerbating existing gut permeability caused by the disease. Researchers should also be cautious in exploring fecal transplants. The approach has worked well for treatment of recurrent *Clostridium difficile* infection that has been poorly responsive to other therapies, but fecal transplantation has not worked well for treatment of gut microbiome-related issues in PLWH.

**Glycomics, Immune Function, and COVID-19**

Based on data from people living with HIV—including those who experience long-term side effects after viral suppression—researchers have focused on studying glycans (chain-like structures of sugar molecules) in COVID-19. Glycans can initiate a strong anti- or pro-inflammatory response and contribute to the development and maintenance of several inflammation-associated comorbidities. The sugars carried by glycans on antibodies (e.g., IgG) also play a role in innate immune function, affecting the ability of antibodies to engage immune cells to fight viral infection. While all of these functions fight viral infection, they can also cause inflammation.

Early research has found that severe COVID-19 was associated with a disrupted plasma glycome. Furthermore, COVID-19 severity was associated with differential antibody Fc-mediated innate immune function; antibodies from hospitalized patients perform complement disposition more than non-hospitalized or control patients. However, patients hospitalized with COVID-19 were less able to elicit phagocytosis than non-hospitalized patients. SARS-CoV-2 antibody titers and neutralization abilities did not fully explain these qualitative differences. COVID-19 researchers determined that complement disposition antibodies correlate positively with plasma inflammation and immune activation markers, while phagocytosis antibodies correlate negatively with plasma inflammation and immune activation markers. Therefore, researchers determined that severe COVID-19 seems to be associated with differential antibody Fc-mediated innate immune function.

Researchers have suggested that the microbiome releases high levels of glycan-degrading enzymes that can translocate and degrade glycans in IgG, in turn significantly disrupting IgG itself. However, researchers have not determined whether the driver of this degradation is an endogenous or exogenous enzyme; this uncertainty has led researchers to pursue metabolome and immune-related research separately.

**Future Research**

Next steps for immune-related COVID-19 research includes determining whether qualitative antibody features impact the function of naturally-induced and vaccine-mediated antibodies in individuals suffering or not suffering from PASC. Preliminary results suggest that SARS-CoV-2 antibodies from people with PASC engage in innate immune functions more than antibodies from people without PASC. Based on these preliminary findings, researchers believe that individuals who develop PASC have lower titers of spike and RBD antibodies but the antibodies that do persist are more likely to initiate complement deposition and phagocytosis via Fc receptors. Researchers are still working to successfully mimic severe COVID-19 and PASC in animal models. Some aspects of these disease states have been successfully recreated; systemic connections of the gut and lung have not yet been replicated for testing potential treatments.
COVID-19 and Diabetes: A Bidirectional Relationship
Franck Mauvais-Jarvis, MD, PhD, Tulane School of Medicine – Delivered October 14, 2021

- Diabetes appears to increase risk of COVID-19-related mortality in a sexually dimorphic way: diabetic women may lose the protective effect of female sex against severe COVID-19 and there appear to be sexually dimorphic diabetes-associated biomarkers of elevated COVID-19 risk.
- COVID-19 also increases risk of new-onset diabetes, which may be an element of PASC.
- Further research is needed to assess whether COVID-19 causes pancreatic dysfunction.
- These findings suggest potential inflammation- and coagulation-related drug development pathways to address both acute COVID-19 and PASC.

Introduction
Research on COVID-19 has focused on the unidirectional impact of comorbidities (e.g., diabetes, obesity) on risk of mortality from SARS-CoV-2 infection. However, little attention has been paid to what diseases and disorders may be caused by COVID-19 infection. Recent research has found that while diabetes is a known risk factor for increased mortality for certain patient populations for COVID-19, COVID-19 may also be causing new onset of diabetes in patients without previous risk factors during acute COVID-19 infection. This finding points to the need for research on the potential role of diabetes in PASC.
COVID-19 and Diabetes Epidemiology

To understand the full scope of the relationship between diabetes and COVID-19 infection, researchers first need to define the most-affected populations. Early in the COVID-19 pandemic, statistics from the New York Department of Health—where one of the greatest surges occurred—suggested that men were more likely than women to be hospitalized and die from COVID-19. In fact, statistics from around the world suggested that men accounted for two-thirds of COVID-19 fatalities. Researchers found another surprising fact: individuals with pulmonary diseases, which are documented risk factors for mortality from other infectious diseases, accounted for a smaller proportion of hospitalized cases of COVID-19 (chronic pulmonary disease 14% and heart disease 18%) than individuals with metabolic disease (obesity 42%, diabetes 34%, and hypertension 57%). These findings held true at other hospitals where patients had different socioeconomic and health status than those in New York City. Dr. Joshua Denson of Tulane University spearheaded a research project at Tulane Hospital and University Medical Center, showing a higher prevalence of mortality in individuals with hypertension, diabetes, and obesity.
Additional research has suggested that sex and diabetes have an interactive association with COVID-19 risk. Mauvais-Jarvis and Coworkers at Tulane examined demographic characteristics and pre-infection comorbidities in COVID-19 male and female patients, they found that individuals with severe COVID-19 had pre-existing obesity, diabetes, hypertension, and pulmonary disease; additionally, women did not experience the previously observed protective effect. A multivariate analysis of the data showed that diabetes was an independent predictor of death only in women. The same was found for chronic kidney disease. These findings also applied when the analysis was stratified by race.

Hypothesized Mechanisms of Diabetes Risk for COVID-19

Researchers have investigated different risk factors associated with diabetes (e.g., blood glucose levels, type of diabetes, BMI) to determine which factors directly correlate with increased risk of mortality from COVID-19. For example, researchers investigated the impact of blood glucose level on COVID-19 outcomes, because blood glucose level is a known risk factor for poor outcomes for other infectious diseases. Type 2 diabetes patients with poorly-controlled blood glucose levels were ten times more likely (11%) to die from COVID-19 that those with well-controlled blood glucose levels.
Type 2 diabetes is associated with obesity, which research has shown is a risk factor for COVID-19 mortality. According to the body mass index (BMI), individuals with a BMI score over 30 are considered obese and individuals with a score over 40 are severely obese. Researchers found a positive linear correlation between BMI score and COVID-19 severity.\textsuperscript{90,91} Researchers believe that one possible reason for the increased risk of severe COVID-19 infection and mortality is the effects of increased body fat exacerbating the cytokine storms caused by COVID-19 infection.

Cytokine release syndrome, more commonly known as cytokine storms, occur when an infection triggers the immune system to flood the body with inflammatory proteins called cytokines. Individuals with higher BMI scores, particularly those who are obese or severely obese, have innate immune cells already producing increased cytokine levels in the blood, creating systemic and chronic low-grade inflammation. This innate inflammation is then further exacerbated by the cytokine storm from COVID-19, making the inflammation, particularly in the lungs, harder to control. Additionally, men have an innate inflammatory immune response; their bodies respond to infection by releasing inflammatory factors, which also complicates treatment of inflammation caused by the cytokine storms.
Future Research

Further research is needed to clarify the mechanisms of diabetes-related risk for severe COVID-19 and to understand the apparent sexual dimorphism exposed by studies of this risk. When researchers focused on specific biomarkers, they found that in males D-dimer levels were a predictor of in-hospital death while in females, ferritin levels and neutrophil lymphocyte ratio (NLR) were both predictors of in-hospital death.

One hypothesis is that (diabetes-associated) lymphopenia may be more lethal in women because it undermines their otherwise stronger immune response, compared to men, to viruses and vaccination. The association of elevated D-dimer levels and COVID-19 mortality in men may indicate that COVID-19-related coagulation is complicating men’s pre-disposition to coagulation and resulting complications (e.g., cardiovascular disease); this risk is itself already exacerbated by diabetes.\(^\text{92}\) These findings emphasize the importance of stratifying risk factors by sex for future COVID-19 research and of identifying the specific causes of mortality in COVID-19 deaths.\(^\text{87}\)

New-Onset Diabetes in COVID-19

Amidst the pandemic in 2020, researchers began to publish papers regarding the new onset of diabetes among hospitalized COVID-19 patients. Alongside these cases of new-onset diabetes, COVID-19 infection was also associated with cases of acute inflammation of the pancreas (pancreatitis), which is responsible for producing insulin. Researchers thus began more closely investigating the effects of COVID-19 on the pancreas, and found that SARS-CoV-2 cell entry factors ACE2 and TMPRSS2 are expressed in the microvasculature, ducts, and islet cells of the human pancreas and that SARS-CoV-2 infects and replicates...
in cells in the human endocrine and exocrine pancreas.\(^{93,94}\) Furthermore, SARS-CoV-2 infection induces beta cell transdifferentiation, causing the beta cells to stop producing insulin, potentially leading to diabetes.\(^{95}\)

Researchers focused on the consequences of SARS-CoV-2 infection in non-human primates (NHP) and human pancreata observed the effects of beta cells and SARS-CoV-2 infection in 4 control and 8 infected NHP and in samples from 6 control and 9 COVID-19 infected human pancreata. Researchers confirmed that SARS-CoV-2 infects pancreatic ductal and endothelial cells in non-human primates and endocrine, exocrine, and endothelial cells in humans. These infections lead to pancreatic thrombofibrosis in NHP and humans, increasing the risk of pancreatic dysfunction. Additionally, researchers found that SARS-CoV-2 viral particles were present in pancreatic cells to be carried throughout the body. By the end of the study, four of the infected primates and two of the infected humans developed hyperglycemia, indicating new-onset diabetes.

**Future Research**

Further research is required to determine the full extent of the impact of COVID-19 infection on the pancreas and its potential relation to new-onset diabetes post-acute COVID-19 infection, as well as to PASC as a whole. A particular area of interest may be the impact of COVID-19 vaccination on protection from new-onset diabetes, pancreatitis, and PASC. A final area of interest is the potential connection between COVID-19 infection in different body systems and organs (e.g., infection rates in the lung and pancreas).
Metformin for Treating COVID-19
Given the connection between inflammation, diabetes, and COVID-19, metformin may prove to be a useful treatment for COVID-19 patients with pre-existing diabetes. Metformin has been used to treat diabetes and has reduced diabetes-related mortality among diabetes patients. A single study from the University of Alabama showed that metformin reduced COVID-19 mortality among patients with pre-existing diabetes. Researchers should consider testing metformin for the treatment of COVID-19 in the NHP setting, given the similarity of the infection and pancreatic dysfunction between NHP and humans.

PASC, COVID-19, and the kidney: a suPAR example
Jochen Reiser, MD, PhD (Rush University Medical Center) - Delivered January 7, 2022

- Animal studies and early human findings suggest soluble urokinase plasminogen activator receptor (suPAR) overexpression is an immune-mediated risk factor for kidney disease.
- SARS-CoV-2 infection is a strong inducer of suPAR.

Defining Kidney Disease
Proteinuria—excess protein in urine due to leakage from the kidney—is a common early sign of kidney dysfunction and is a risk factor for both renal and extrarenal disease and mortality. End-stage renal disease is one of the most expensive medical conditions, accounting for over $32 billion and more than one quarter of Medicare’s expenditures annually. The number of patients affected and the associated treatment costs are expected to continue to rise in coming years, in part due to the increased incidence of renal disease in patients with COVID-19 and PASC.

Historically, research on renal disease therapies is lacking compared to research for diseases with a similar burden. Part of this deficit is due to the complexity of the renal system biology, which includes 26 or more different cell types. During the last decade, however, the identification of surrogate endpoints for chronic kidney disease (CKD), such as proteinuria, have enabled some progress in preclinical and clinical research. Dr. Reiser described three categories of CKD biology and treatment: physiological, structural, and immunologic. One physiological factor related to the progression of CKD is blood pressure, which has a direct relationship with CKD progression. Structural (i.e., genetic) components of CKD are increasingly identified and may be the target of genetic therapies in the future. Immunologic components of CKD, particularly acute and chronic inflammation, can have a major impact on CKD onset and progression, especially because inflammation in CKD is often treated generically with steroids until side effects preclude that approach. Researchers have determined that if the immune system that interacts with the kidney could be treated specifically, CKD could be better treated.

Specific anti-inflammatory treatments of acute kidney injury are emerging considerations as well backed by a growing literature of novel biomarkers and risk factors which suPAR being one of them.

Immune-Pathobiology of the Kidney
Podocytes—specialized cells that act as part of the dynamic filtration unit in the kidney—are cells in that serve as immune targets in the kidney that can fail in the presence of chronic inflammation, resulting in
proteinuria. Podocytes are negatively affected by inflammation or increased blood pressure, first in the form of effacement and eventually cell death. These effects lead to disturbances in the kidney filtration system—an early indicator of kidney disease. The body can tolerate an approximate loss of 20% of podocyte cells in the filtration system before scarring occurs, leading to kidney dysfunction.

The earliest podocyte damage is effacement—the shortening and fusing of the foot processes on podocyte cells. Effacement increases the porousness of the filtration system and allows protein to transfer from blood to the urine more easily. If effacement is not reversed, podocytes continue to deteriorate, leading to foot detachment and resulting in irreversible scarring of the foot and cell.

Given the importance of effacement to kidney deterioration, researchers have focused on finding biomarkers of the process, which can be measured only via electron microscopy. Dr. Reiser and his team found that when nephrotoxins were attached to highly differentiated podocytes in vitro, motility of the cells measurably increased. When these cells were then treated by removing key regulators of motility, their motility decreased or stabilized. Motility is a surrogate for failing and healing podocytes.

In the search for additional surrogates for podocyte effacement, researchers found that activation of integrin systems also changes podocyte foot process motility. Integrins are cell signal regulators that are responsible for the activation of cell processes that lead to effacement. Integrins may be activated by receptors such as urokinase plasminogen activator surface receptor (uPAR) on cells throughout the body or soluble uPAR (suPAR) in the bloodstream. The binding of suPAR to podocytes activates the processes for effacement.

**Role of uPAR and suPAR in the Immune and Renal Systems**

uPAR is a three-finger protein that is expressed naturally at around 2 ng/mL in humans (2.4 ng/mL in women and 2.2 ng/mL in men) as an innate immune messenger. However, at elevated levels, uPAR and suPAR can become toxic, contributing to chronic inflammation and CKD. Dr. Reiser shared a case study from 2018 of identical twins, one of whom was reportedly bitten by a brown recluse spider. After severe effacement and loss of the kidneys, the affected twin received a transplanted kidney from the sibling; however, shortly after transplant the effacement and failure recurred. At the time of recurrence, the affected twin had massive proteinuria and high suPAR levels. suPAR is created mainly in the bone marrow, from immature myeloid cells that are stimulated by viruses or toxins (e.g., SARS-CoV-2). suPAR then travels through the blood to distant organs, leading to manifestations such as acute kidney disease. In fact, increased suPAR levels and the manifestations of increased suPAR have been observed in acute severe COVID-19 patients and have begun to be observed in patients with PASC. Researchers believe that inhaled viruses, such as SARS-CoV-2, may be particularly stimulating of increased uPAR and suPAR expression because they trigger increased protein expression in the bronchial tree in the lungs, but the underlying processes are not fully understood.
Researchers have not yet determined the exact mechanism that triggers the body to release suPAR. During acute infection, the spike protein of a virus (e.g., SARS-CoV-2) may allow for the strong activation of innate immune cells. In addition, the body produces suPAR to help remove large protein complexes that are generated during viral infection and that cannot be naturally eliminated through kidney filtration. However, during long-term infection, suPAR continues to be produced and becomes maladaptive within the body.

**suPAR as an Effacement Surrogate**

suPAR can be reliably measured using two available ELISA assays. Furthermore, suPAR has been found to be correlated with severe kidney disease, including focal segmental glomerulosclerosis (FSGS) and recurrence after transplant. Researchers found that in FSGS patients, those with higher suPAR in the blood were at greater risk of recurrence post-transplant.
To further study this connection, researchers humanized mouse models using peripheral blood mononuclear cells from FSGS patients who had high suPAR levels and experienced recurrent disease. After about 10 weeks, the humanized mice developed proteinuria and effacement whereas the control mice who were humanized with healthy PBMCs did not. Based on these findings, researchers have continued to study uPAR and suPAR levels in humanized mice as a modulator of integrin function and a potential pathway for treatment of renal disease. Dr. Reiser shared the results of a study illustrating the activation of integrin and resulting effacement at the onset of kidney disease. Researchers also conducted a study showing that in mice modified to be incapable of activating integrin, suPAR does not cause effacement. Studies in mice also showed that a stronger binding variant of suPAR—that upon characterization was illustrated to be a dimer—was associated with greater incidence of proteinuria and kidney disease. However, when mice infected with the stronger binding variant are crossed with integrin knockout mice, and the mice are denied the integrin activation, the mice can be rescued.

These findings in mice hold true in human models, as well. Single cell RNA sequencing in early human diabetic nephropathy showed upregulation of podocyte integrin beta3—the suPAR receptor. Further studies in humans have shown that suPAR is the strongest independent predictor of decline of kidney function in patients with kidney disease, and is 38% accurate at predicting CKD diagnosis within 5 years in patients that did not yet have kidney disease. Combining suPAR with other markers (e.g., hs-CRP) could improve the risk prediction for kidney disease. The ability of suPAR to predict kidney disease was validated in Swedish and Chinese studies. Another study found that if African American individuals with a genetic risk factor for kidney disease did not have high suPAR levels, the annual loss of renal function was almost equivalent to individuals without a genetic risk factor. Similar findings have been observed in patients with sickle cell disease and autosomal kidney disease. In summary, individuals with healthy kidneys that have low suPAR are at low risk for developing kidney disease; individuals with kidney disease and low suPAR levels are at low risk for progression of disease; individuals with individuals with healthy kidneys with high levels of suPAR are at high risk of developing kidney disease; and individuals with kidney disease and high suPAR levels are at high risk for progression of disease.
suPAR and COVID-19

Similar to hospitalized patients with other acute infections (e.g. sepsis), patients hospitalized with COVID-19 have been shown to develop acute kidney injury (AKI). Based on the studies above, researchers sought to determine whether suPAR impacted the development of AKI in COVID-19 patients. They found that patients with high suPAR levels who are admitted to the hospital are more susceptible to AKI than those with low levels of suPAR, independent of other risk factors such as pre-existing kidney disease or diabetes. Recently, a study examining patients admitted for acute COVID-19 found that if suPAR levels rose above 6.86 ng/mL, the individual was at dramatically increased risk of developing AKI compared to those with lower suPAR levels.

Researchers further studied AKI and suPAR using transgenic mice injected with an anti-suPAR antibody and found that the ability for suPAR to develop and bind greatly influenced the development of AKI. This finding suggests that suPAR is a part of AKI’s causal pathway.

The International Study of Inflammation in COVID-19 (ISIC) was undertaken to observe outcomes for individuals hospitalized for COVID-19. Patients in ISIC who had proteinuria were found to have a median suPAR level of 10.42 ng/mL and an incidence rate of AKI of 70.3% while individuals who did not have proteinuria were found to have a median suPAR level of 6.71 ng/mL and an AKI incidence rate of 31.4%.

A study of COVID-19 patient biopsies found that patients experienced podocytopathy, immune-mediated glomerular diseases, tubulo-interstitial disease, and allograft pathologies even when there was no definitive detection of COVID-19 virus in the kidney. These findings favor cytokine-mediated effects and heightened adaptive immune responses as the reasons for kidney-related issues in COVID-19 patients. However, another study showed that the SARS-CoV-2 virus does appear in kidney cells, suggesting that it may directly contribute to pathology that researchers need to further investigate. Researchers propose that the collapsing glomerulopathy associated with COVID-19—termed COVID-19 associated neuropathy (COVAN)—affects individuals with risk factors for an inflammatory immune response to SARS-CoV-2.
respiratory infection. To test this hypothesis, researchers studied suPAR mice, uPAR knockout mice, and control mice that inhaled human spike protein of SARS-CoV-2 and found that after 11 days the knockout mice did not react to the spike protein but the mice with high suPAR levels that received the spike protein had an intense kidney reaction that led to early stages of kidney disease (i.e. proteinuria). Next steps for researchers include performing clinical trials to assess suPAR’s potential as a therapeutic target to prevent development and progression of kidney disease.

Although AKI is the main renal issue seen in acute COVID-19 infection, it is not unique to COVID-19 patients but is in fact typical in patients who are hospitalized for other infections (e.g., sepsis). Similarly, renal issues identified in PASC patients are similar to transplant glomerulopathy—immunomodulation that causes changes in pathology due to persistent activation of inflammatory pathways. Renal changes in PASC patients will likely be a direct result of this persistent inflammation and virus activity in kidney cells. Now that more patients have been living with PASC for an extended period of time, researchers need to turn their attention to these issues.

Because suPAR is a marker of chronic inflammatory processes, researchers have also seen indications that suPAR levels are good predictors of acute lung injury in COVID-19 and long-term COVID-19 related lung outcomes; however, more research is needed to understand the extent of the correlations and the mechanism of action driving them.

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Observations on Long COVID Through a Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) Lens

Peter Rowe, MD, (Johns Hopkins School of Medicine) - Delivered January 14, 2022
• ME/CFS is known to develop in a gradual, insidious manner in some, but more commonly to occur following a variety of acute infections.
• The symptom overlap between PASC and ME/CFS suggest the lessons learned from ME/CFS treatment research—in particular, treatments related to orthostatic intolerance—may also inform therapeutic research on PASC.
• The fact that many PASC patients who had mild or asymptomatic COVID-19 experience symptoms characteristic of ME/CFS and its comorbid conditions raises the question whether some forms of ME/CFS that were considered “noninfectious” in the past might have been sequelae of mild or undetected infections.

Introduction
Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) occurs in 10-13% of individuals 6 months after acute infection with infectious mononucleosis. The severity of the acute mononucleosis infection is the main risk factor for the development of ME/CFS. Approximately 25-40% of patients with ME/CFS experience a gradual, insidious onset of symptoms, without a clear initiating infection or event. Recently, similarities between ME/CFS and PASC—including a low incidence rate in children under 10 years of age, a substantial overlap in symptomatology, and because PASC can develop in individuals who experienced mild or asymptomatic acute COVID-19 infections—have led ME/CFS researchers to consider whether an initiating infectious agent might have triggered a proportion of insidious-onset cases.

Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS)
The quality-of-life impact of ME/CFS in adults is equivalent to that of multiple sclerosis and chronic heart failure, with many adult patients disabled as a result of their ME/CFS. Pediatric ME/CFS is one of the most common causes of prolonged school absence in developed countries, and the main risk factor affecting attendance is reduced physical function. In several quality-of-life surveys, children with ME/CFS have lower function than children attending clinics for cystic fibrosis, sickle cell disease, epilepsy, and other common pediatric chronic illnesses.

Females have an approximately 3-fold higher risk for developing ME/CFS than males. ME/CFS has a heritable component: it is more likely to occur in members of the extended family. Twin studies show a higher concordance for the diagnosis in monozygotic versus dizygotic twins. ME/CFS is also more common in patients with Ehlers-Danlos Syndrome, a heritable disorder of connective tissue characterized by varying degrees of skin laxity, joint hypermobility, and delayed healing. In a study of 58 consecutive adolescents with ME/CFS, the odds of having joint hypermobility were 3.5 times higher than in a similar number of age- and sex-matched controls. Among those with ME/CFS, 60% had a Beighton score for joint hypermobility of at least 4, compared to 24% of healthy controls, indicating that joint hypermobility is a physical risk factor present since birth.

Randomized controlled trials of pharmacologic treatment for ME/CFS have not identified a single effective pharmacologic agent, but standard forms of symptomatic therapy can be effective for comorbid conditions (e.g., pain, menstrual dysfunction, migraines, orthostatic intolerance, sleep problems, and biomechanical dysfunction).
Dr. Rowe’s group has been interested in the high prevalence of biomechanical problems in those with ME/CFS, even though many have joint hypermobility. In a study of 11 physical therapy measures of range of motion of the limbs and spine, ME/CFS patients were more restricted than healthy controls, and had statistically significant restrictions on 6 of those measures. However, researchers are still unsure of the mechanism for the adverse restricted range of motion and non-compliance of the nervous system. Researchers have determined that range of motion issues are not permanent. ME/CFS patients were worse at baseline compared to healthy controls but were able to improve range of motion scores dramatically by 24 months after exposure to multi-modal therapy that often included manual physical therapy.
Orthostatic Intolerance, ME/CFS, and PASC

Orthostatic intolerance seems to be due to increased pooling of blood in the peripheral circulation along with a reduction in intra-vascular volume, leading to a reduced return of blood to the heart and a decrease in cerebral blood flow when individuals are upright. This decreased cerebral blood flow leads to an increase in sympatho-adrenal response.

Common forms of orthostatic intolerance include postural orthostatic tachycardia syndrome (POTS) and neurally-mediated hypertension (NMH), and increasingly research in adult ME/CFS patients shows that orthostatic intolerance can be present even when heart rate and blood pressure remain normal during upright tilt table testing. Researchers have shown that a tilt test is capable of provoking fatigue and that orthostatic intolerance is strongly associated with both chronic fatigue and ME/CFS. Both adolescents and adults with ME/CFS respond to open treatment of orthostatic intolerance, with one study showing baseline entry wellness scores averaging 35 out of 100 and improving after 4 months of treatment to an average of 70 out of 100. Therefore, while an incomplete treatment, therapy to address orthostatic intolerance is effective in helping improve function in patients with ME/CFS.

Dr. Rowe hypothesized that joint hypermobility confers an increased risk of ME/CFS due to the fact that the same connective that contributes to lax ligaments and stretchy skin is present in the connective tissue of the blood vessel wall. The vessels are therefore more likely to dilate in response to hydrostatic pressure in the dependent limbs, leading to increased peripheral pooling of blood, and less blood return to the heart and brain.

Over 95% of pediatric ME/CFS patients have evidence of orthostatic intolerance. In the 2015 Institute of Medicine summary of the published literature, there was a wide 0-96% range in the prevalence of orthostatic intolerance among adults (mean 42%). Using transcranial Doppler imaging, cerebral blood flow velocity in 23 controls and 26 ME/CFS patients identified no statistical differences between groups, despite a much higher prevalence of orthostatic symptoms during tilt in those with ME/CFS.

Researchers Linda van Campen and Frans Visser in the Netherlands introduced new method of measuring total cerebral blood in-flow. They calculated flow through both internal carotid and both vertebral arteries using a Doppler probe. After seeing that the technique was accurate and reliable in healthy patients, they applied the technique to a large sample of 429 ME/CFS patients and 44 healthy controls.
ME/CFS patients, 58% had a normal heart rate and blood pressure response to 30 minutes of head-up tilt, but nonetheless experienced an average of 24% reduction in cerebral blood flow according to Doppler echography. By comparison, healthy control volunteers experienced an average reduction of 7%. Another 14% of the ME/CFS patients had delayed orthostatic hypotension, and 28% had postural tachycardia syndrome, and these patients had 28% or 29% reductions, respectively, in cerebral blood flow. The percent of cerebral blood flow reduction correlated with the number of symptoms patients reported. In total, 90% of adults had orthostatic intolerance as measured by the cerebral blood flow measures, suggesting this is a more sensitive means of detecting clinically important physiologic changes than simply relying on heart rate and blood pressure.

These researchers extended the Doppler echography method of measuring cerebral blood in-flow to the brain to 10 consecutive adult PASC patients with a disease duration ranging from 6 to 15 months. As controls, the researchers also included 20 ME/CFS patients with POTS, 20 ME/CFS patients with normal heart rate and blood pressure response to head-up tilt, and 20 healthy controls. ME/CFS and PASC patients had no statistical differences in most symptoms, including fatigue, post-exertional malaise, cognitive dysfunction, headache, sensory hypersensitivity, sore throat, and respiratory symptoms. On the Doppler readings, PASC patients experienced an average 33% reduction in brain blood flow, compared those with ME/CFS and POTS who experienced an average 29% reduction, ME/CFS patients with normal blood pressure and heart rate responses who experienced an average 25% reduction, and healthy controls who experienced an average 4% reduction. In addition, all PASC patients met criteria for both ME/CFS and POTS.

The Doppler echography of the extracranial vessels is not available widely yet in clinical settings. Performing the Doppler echography requires training, but in experienced hands the measurements have high reliability and inter-observer agreement. Testing all PASC patients for these symptoms is unlikely to be available clinically in the short term, but may be a valuable method in research settings.

**ME/CFS Lens for PASC in Adolescents and Young Adults**

Researchers have found that adolescent and young adult PASC patients appear to have some of the same risk factors as adolescent and young adult ME/CFS patients. For example, in a case series of three patients between the ages of 19 and 30, all three patients met the Institute of Medicine criteria for ME/CFS by 6-months after COVID-19. In this group of patients, all had florid POTS during a 10-minute passive standing test, with a peak heart rate ranging from 129 to 166 beats per minute. Additionally, each of the patients developed their orthostatic symptoms within the first two weeks of the onset of acute respiratory symptoms. Two patients had pathologically brisk reflexes, and two had positive Hoffman signs, suggestive of pathological neurological irritability. One met the criteria for hypermobile Ehlers Danlos Syndrome, and all experienced increased allergic or inflammatory manifestations.

Dr. Rowe presented findings specifically from a 19-year-old patient as an instructive example of the similarities between PASC and ME/CFS. The patient has a history of Gilbert’s syndrome, allergies to pollens and grass, a persisting oral allergy to carrots, cashews, and cherries, and mild asthma. Before his confirmed COVID-19 illness, he attended university and ran 60-70 miles weekly as a member of the cross-country team. In June 2020, he was exposed to COVID-19 and experienced mild upper respiratory
syndrome (e.g., cough, sore throat, headache, fatigue). His sense of smell was abnormal for several months, but he was never hypoxic nor hospitalized for the acute infection.

Two weeks after the onset of COVID-19 symptoms, he attempted to return to his running routine, but experienced coughing, labored breathing, and lightheadedness, as well as post-exertional malaise. In addition, he experienced an elevated heart rate while walking between rooms at home. Two months after onset, while playing a beanbag game, he experienced an elevated heart rate of 170 beats per minute for 30 minutes, followed by 3 days of post-exertional malaise. Troponin levels, chest x-ray, echocardiogram, and cardiac MRI were all normal. When evaluated in person, he had a limited 35 degree range of motion on passive straight leg raise, brisk reflexes and a bilaterally positive Hoffman sign, elevated plasma histamine levels, and a 70 beat per minute increase in heart rate on a 10-minute passive standing test.

Medications typically used to treat POTS (e.g., fludrocortisone, midodrine, pyridostigmine bromide, ivabradine) were ineffective or not tolerated. Eventually, he gained a modest benefit from a combination of clonidine, methylphenidate, escitalopram, and low-dose naltrexone. After 12 months of treatment, he was able to complete a 40-hour/week virtual summer internship. One year after onset, his main symptoms were fatigue, unrefreshing sleep, and post-exertional malaise. He could still tolerate only a 15-minute walk twice daily, with a heart rate rising to the 130-140 bpm range. After 18 months post-COVID-19, he was able to attend in-person university courses and started manual physical therapy to address the movement restrictions. He added stationary biking, advancing the duration and intensity gradually to avoid provoking post-exertional malaise. For example, in September 2021, the individual was able to ride the stationary bike for 15 minutes twice per week, and by January 2022, could ride for 30 minutes four times per week.

This single patient illustrates some of the similarities between ME/CFS and PASC patients. Researchers should thus apply learnings from treatments that did or did not work for ME/CFS to the treatment of PASC—especially since POTS and orthostatic intolerance are among the most treatable conditions.
associated with ME/CFS. Further research is needed to understand the underlying mechanisms contributing to the similar symptoms between ME/CFS patients and PASC patients. Preliminary research points to the expression of ACE receptors in the brain stem and may indicate that virus binding to that area could negatively affect autonomic function. Alternatively, the virus may be triggering auto-immune inflammation and unmasking underlying comorbid conditions.

Researchers will also need to determine the exact influence of COVID-19 vaccinations on individuals who develop PASC. Early anecdotal evidence has shown that some patients with POTS and PASC experience improvement post-vaccination while others have experienced marked decline and increased symptoms post-vaccination. Determining what causes some patients to react well and others to react poorly could potentially inform vaccination plans for future patients, and might provide insights into the pathophysiology of general symptoms.

**Machine Learning Algorithm for Diagnosing Pediatric Patients with Multisystem Inflammatory Syndrome**
*Jane Burns, MD (UCSD School of Medicine) and Shamim Nemati, PhD (UCSD) - Delivered January 28, 2021*

- Like PASC, multisystem inflammatory syndrome may occur in children (MIS-C) or adults (MIS-A) after an acute infection from COVID-19.
• To help differentiate diagnosis of MIS-C from other febrile conditions in pediatric patients, UCSD researchers developed a machine learning model that identified clinical and laboratory patterns in MIS-C patients.

Introduction
As early as April 2020, physicians in the United Kingdom began reporting cases of children currently or recently infected with COVID-19 developing symptoms of a secondary, febrile condition. In early May 2020, the New York State Department of Health reported cases of the same condition. Shortly thereafter, the Centers for Disease Control and Prevention (CDC) and World Health Organization (WHO) released case definitions for the febrile condition *multisystem inflammatory syndrome in children* (MIS-C). The CDC *case definition* is an individual under the age of 21 years old presenting with fever of greater than 38°C for greater than 24 hours, laboratory evidence of inflammation, evidence of clinically severe illness requiring hospitalization with multisystem organ involvement, and positive for current or recent SARS-CoV-2 infection or COVID-19 exposure within 4 weeks of onset of symptoms. It is unclear what underlying pathophysiological mechanisms cause MIS-C to occur. Furthermore, diagnosis of MIS-C in the field is complicated by its clinical similarities to other febrile conditions (e.g., Kawasaki disease).

MIS-C and Kawasaki Disease
Neither MIS-C nor Kawasaki disease have a single diagnostic test, but instead present with a pattern of clinical features and laboratory abnormalities, some of which overlap. For example, both Kawasaki disease and MIS-C are associated with bloodshot eyes, swollen lips, and rash. In early 2020, this overlap led many physicians to raise concerns about possible outbreaks of Kawasaki disease that later were confirmed to be MIS-C, and the conditions continue to be confused by frontline physicians who are unfamiliar with either or both conditions given their rarity. In the United States, over 6,400 cases of MIS-C have been reported as of January 2022, and annually approximately 6,000 cases of Kawasaki disease are reported each year.
Some of the features that differentiate MIS-C and Kawasaki disease patients include age, GI symptoms, ethnicity, and laboratory parameters (e.g., white blood cell count). For example, children of African American and Hispanic descent are overrepresented among MIS-C patients, while children of Asian descent are overrepresented among Kawasaki disease patients. Based on these patterns, Dr. Burns compared the University of California, San Diego (UCSD) database of Kawasaki disease and MIS-C patients and found that decreased white blood cell count, decreased platelets, and decreased sodium were all common laboratory findings for MIS-C patients but each only occurred in only 2 percent of Kawasaki disease patients. Recognizing the potential for pattern recognition to help differentiate diagnosis of MIS-C and Kawasaki disease, Dr. Burns engaged bioinformatics machine learning experts at UCSD to create a partnership to design an artificial intelligence approach to MIS-C diagnosis.

**MIS-C Diagnosis Algorithm**

Dr. Nemati and his team, led by PhD candidate Jonathan Lam, created a machine learning model that can make a prediction about whether a patient has MIS-C, Kawasaki disease, or another febrile condition. The model is an artificial neural network (ANN)—a machine learning model inspired by the hierarchies in the primary control visual cortex. ANNs have shown high performance capabilities in industrial applications (e.g., voice recognition) and are good at identifying multiplicative risk factors (e.g., the interaction between immunosuppression and hypothermia in the presence of an infection may be indicative of risk).

To determine whether a patient has MIS-C, Kawasaki disease, or another febrile condition, the algorithm uses data from 17 laboratory measurements and 5 clinical signs to perform a two-stage assessment: first, it determines whether a patient is at risk for MIS-C and then, if the patient is not at risk for MIS-C, whether they are at risk for Kawasaki disease or another febrile condition. Each of the 21 total data points is assigned an odds ratio, which indicates increased or decreased risk for each of the two diseases; smaller odds ratios are associated with reduced risk and higher odds ratios are associated with increased risk. Based on a patient’s symptoms, the model applies the odds ratios to calculate a prediction of whether the patient has MIS-C, Kawasaki disease, or another febrile condition. This prediction can be utilized by physicians to guide their diagnostic testing and ultimately their clinical diagnosis. The input from the physician is critical, because the algorithm may not have...
access to all the patient history, clinical signs, and multiplicity of risk factors that may underlie a patient’s symptomology.

Validating the Machine Learning Model to Identify MIS-C

Machine learning models like the one Dr. Nemati’s team developed must be fed reliable, high-quality data to identify the patterns that will inform the assignment of odds ratios used in determining whether a patient is at risk for MIS-C. To ensure that the algorithm was created using quality data, the researchers leveraged a training cohort of 673 febrile pediatric patients, 775 Kawasaki disease patients, and 131 MIS-C patients from three hospitals. The team first used 80% of the data available to train the model in pattern recognition. The gold standard diagnosis to train the algorithm was prospective diagnosis by pediatric specialists for Kawasaki disease following American Heart Association guidelines or MIS-C following CDC guidelines. Then, using patterns identified in the symptomologies of the training cohort patients, the model calculated odds ratios for the data points (i.e., the 17 laboratory results and 5 clinical signs). The model was then tested on the remaining 20% of the data.
The next step in preparing a machine learning model is to validate the training using an external validation process. To externally validate the model, the team used a cohort of 175 MIS-C patients from 16 hospitals around the United States. The sensitivity of the model ranged from 90% accuracy for predicting MIS-C in patients from Washington DC to 95.9% accuracy in predicting MIS-C in patients from Boston.

Based on the immediate need of clinicians, the team sought to bring the model to a prospective setting. The machine learning team has developed a website that utilizes the model to allow clinicians to input data from their patients into a risk calculator that then uses the model to predict whether a patient is at risk for MIS-C. The website is currently restricted to UCSD use, but the team hopes to open it for wider use in the future. The machine learning team emphasized that the tool cannot replace the role of physicians in diagnosing MIS-C, Kawasaki disease, or other febrile conditions as it is not able to account for factors outside the data input.

Over the past several years, biased datasets have been a key concern in the development of machine learning models. One of the main sources of bias is lack of diversity in the training dataset, which causes a model to work better on some patient populations than others. Building a diverse training population that leverages national datasets, as was done for this project, helps to mitigate the bias of the model. To further address bias the machine learning experts included a boundary around the algorithm, which enables the model to identify whether it has enough prior knowledge of similar cases to reliably determine whether a patient is at risk for MIS-C. If the algorithm finds the data lacking, it will flag the case as an indeterminate result. These flags reduced false alarms for similar machine learning models by 75 percent. This reduction of false alarms can greatly reduce the burden of resources or time spent on a false diagnosis.
Utility of the Machine Learning Model
Within the populations of patients being differentiated by the machine learning algorithm, the MIS-C and Kawasaki disease patients require fast diagnostic and treatment decisions due to the life-threatening nature of their illnesses, whereas the febrile controls are often able to recover without acute treatment. Therefore, the diagnostic model is a useful clinical support tool that allows clinicians to input data from low-cost, fast-response laboratory tests in addition to clinical impressions and receive assessments to guide their subsequent diagnostic measures. For example, if the model suggests a high risk of MIS-C in a patient, the clinician may choose to move forward with more expensive diagnostic testing for MIS-C, whereas if the model suggests a low risk for MIS-C, the clinician may choose to first perform further diagnostic tests (e.g., urine values) to determine whether the patient may have a different source of illness (e.g., kidney infection).

The model’s identification of patterns and relationships may also help researchers to understand the underlying pathophysiology of MIS-C. To help clinicians start to better determine the underlying pathophysiology of MIS-C, the machine learning team utilized advanced interpretability methodologies to produce the risk scores used by the model and to identify the model’s top contributing variables.
Leveraging Multidisciplinary Teams in Research

The advanced diagnostics work enabled by the machine learning model for MIS-C diagnosis would not be possible without a multidisciplinary research team. Dr. Burns stressed the importance of leveraging different perspectives, particularly for disease areas that are not well understood (e.g., Kawasaki disease, MIS-C, PASC), to move research towards better diagnostics and treatments. The close partnership between machine learning group and clinicians also allowed for quality feedback that improved the processes and outputs of the MIS-C diagnostic model. At this time, the feedback on the tool is limited to clinicians partnered with UCSD; however, even among those hospital staff, the project has attracted more collaboration across departments, which can sometimes be siloed in the hospital setting.
Identifying Factors that Put Patients at Risk of Long COVID

James Heath, PhD, President and Professor, Institute for Systems Biology – Delivered March 2, 2022

- A study team from the Institute for Systems Biology (ISB) assessed PASC patients to determine (1) what are risk factors for PASC, (2) at what point in the disease course can these risk factors be assayed, and (3) whether these risk factors are independent or related.
- Clinical conditions associated with higher prevalence of PASC include comorbid conditions (e.g., diabetes), comorbid viruses (e.g., Epstein-Barr Virus viremia), and autoantibodies.
- Co-dependence of these PASC risk factors decreases over time from COVID-19 diagnosis throughout acute infection, and is nonexistent at convalescence, indicating that further research is needed to determine whether treatment during acute infection may change the course or severity of PASC.

Introduction

PASC (i.e., Long COVID) is a chronic condition that occurs after acute infection with COVID-19 and commonly manifests with a variety of symptoms, including brain fog, dyspnea, cough, erythematous or urticarial rash, depression, post-traumatic stress disorder, heart palpitations, fatigue, and myalgia.\(^{131}\) PASC is one of several post-acute conditions that manifest with vague and varied symptomology and for which treatments are sparse. Given the widespread impact of PASC, researchers have been working to better characterize the condition in order to develop effective treatments.

Dr. Heath shared findings from a recently published study by the Institute for Systems Biology (ISB), which assessed PASC patients to determine (1) what are risk factors for PASC, (2) at what point in the disease course can these risk factors be assayed, and (3) whether the risk factors are independent or related.\(^{131}\)

To accomplish this goal, researchers at ISB collected symptoms patients experienced at convalescence after recovery from acute COVID-19 infection, identified risk factors to predict likelihood that an individual would develop PASC, and determined whether there any of these risk factors are immunological and/or whether the risk factors can be used to define therapeutic opportunities.

To address these questions, the research team performed comprehensive immunophenotyping of a large patient cohort looking at three time points: date of COVID-19 diagnosis, one week after diagnosis, and 10-14 weeks after diagnosis. The final collection timepoint captured patients after they recovered from acute infection, but potentially before PASC symptoms fully developed. The study team collected patient-reported symptoms using questionnaires and validated them using electronic health records for all collection timepoints. The study participants’ symptom trends followed those reported by other epidemiological studies. Dr. Heath presented numerous categories of results outlining detailed PASC symptomologies, noting that all results are corrected for age, sex, and disease severity.
Comorbid Conditions
One analysis the study team conducted focused on correlations of PASC symptoms with pre-existing conditions and clinical laboratory findings. The comorbid condition with the strongest correlation to PASC symptoms was Type 2 diabetes, which positively correlated with cough, fatigue, respiratory viral levels, and neurological symptoms. Dr. Heath suggested that this correlation may reflect a causal relationship between diabetes and PASC. Although the study team did investigate whether blood glucose levels during acute infection affected the severity of PASC, the findings were inconclusive and may have been confounded by the variety of COVID-19 treatments used throughout the course of the study. Glycolysis is known to play a role in other risk factors for PASC, but it is unknown whether this role is directly tied to diabetes. The connection between glycolysis and PASC is one area for future PASC research.

Plasma-Based Biomarkers
In response to patient reports of difficulty obtaining primary care for their symptoms, the study team sought to identify plasma-based biomarkers that correlate with PASC symptoms that could be used as diagnostic indicators and potentially identify treatment pathways. To accomplish this identification, the study team generated and analyzed data from patient plasma for protein or metabolomic biomarkers that associate with specific PASC symptoms at the convalescent timepoint 10-14 weeks after COVID-19 diagnosis. The team found evidence of proteomic and/or metabolomic biomarkers for neurological and respiratory symptoms of PASC.

More specifically, the team found evidence that two proteins correlate significantly with an altered circadian rhythm in patients with neurological PASC symptoms. No other proteomic or metabolic signatures were present for neurological PASC symptoms. The team also found evidence of repressed cortisol plasma levels that correlate with respiratory PASC symptoms. Low cortisol, or Addisonian crisis, is commonly associated with extreme fatigue, weight loss and decreased appetite, low blood pressure, and acute adrenal failure. Addisonian crisis is typically treatable through cortisol replacement therapy, which may be a potential treatment for PASC patients with low cortisol, as well. However, Dr. Heath noted that these findings may be confounded if patients are on steroids, which are chemically similar to cortisol and can alter cortisol regulation.\(^{(132)}\)
Comparing Comorbid Viral Loads
A recent publication noted that individuals who were Epstein-Barr Virus (EBV) antibody positive—meaning that they had an EBV infection at some point during their lives—were more prone to developing PASC than those without EBV antibody positivity. Based on these findings, the study team tested their study participants for both EBV antibodies and Cytomegalovirus (CMV) antibody positivity—the two most common latent viruses in the United States—and for EBV and CMV viremia in the blood.

While the team did not find evidence of CMV reactivation, the team did find EBV viremia in approximately 25% of patients at time of COVID-19 diagnosis, a 3- to 4-fold reduction by the 1-week post-diagnosis timepoint, and almost no patients at the 10- to 14-week post-diagnosis timepoint. Dr. Heath also noted that SARS-CoV-2 RNAemia at the time of diagnosis is associated with survival probability. When the study team compared EBV viremia and SARS-CoV-2 RNAemia at COVID-19 diagnosis with PASC symptoms, they found that both are associated with neurological symptoms and fatigue (the association of SARS-CoV-2 RNAemia with this symptomology persisted even after adjustment for COVID-19 severity). Therefore, detectable levels of either virus in the blood at COVID-19 diagnosis may predict PASC development.

Autoantibodies and COVID-19
Autoantibodies have been studied extensively for their association with acute COVID-19 infection and may also be associated with PASC. For example, the presence of certain type 1 interferons can impact a patient’s acute COVID-19 disease trajectory. In fact, type 1 interferons (IFNs) are known immunoregulatory cytokines that activate autoreactive B cells. In a recent publication, researchers identified atypical memory B cells associated with a number of disease conditions, including HIV, HBV, HCV, and lupus. Atypical memory B cells mature to form immunoglobulin (IgG) autoantibodies, but this maturation takes place outside the normal tissue process that occurs within B cell follicles. Some studies have found IgG autoantibodies in COVID-19 patients.

When the ISB study team performed an autoantibody panel on study participants’ samples, they found that individuals with SARS-CoV-2 antibodies were likely to also have other autoantibodies. Moreover, the data showed that higher levels of autoantibodies were correlated with lower levels of protective antibodies against SARS-CoV-2, suggesting that people with elevated autoantibodies are more susceptible to developing breakthrough infections. The ISB researchers also found that patients with lupus autoantibodies at convalescence also had mature autoantibodies at the time of diagnosis, suggesting that these autoantibodies are present prior to COVID-19 infection and do not associate with disease severity, but do associate with the number of atypical memory B cells present. Often, the ISB study patients had autoantibody levels below the limit of detection on typical laboratory testing, but the autoantibodies tended to be functional even below clinical levels. Furthermore, the autoantibodies were associated with
PASC symptoms, including sputum, gastrointestinal and respiratory symptoms, and inability to exercise. This association with symptomologies shows that autoantibodies at the time of COVID-19 diagnosis are risk factors for developing PASC, with higher levels of autoantibodies associated with stronger symptoms.

**Single Cell Analytics and Immune Endotypes**

The ISB study team also performed single cell integrated analytics on the study population, including single cell T-cell receptor sequencing and single cell RNA sequencing (RNAseq). The study team used the integrated T-cell receptor analytics to track how T-cells evolve over the course of the disease and recovery. Certain T-cell receptors identified at diagnosis were associated with their T-cell phenotype, and then are tracked throughout the evolution of acute disease and into recovery. Typically, researchers expect many T-cell clonotypes to form a naïve pool and, as infection increases, to become effectors before contracting into a memory phenotype pool. However, the study team found that this expected process did not occur in all COVID-19 patients.\(^{131}\)

In partnership with Adaptive Biotech, and by leveraging public databases, the ISB study team investigated whether they could identify T-cells specific to SARS-CoV-2 infection or other viruses. The study team identified 150,000 T-cell clonotypes in the patient population that could be assigned to some antigen associated with SARS-CoV-2 and compared the findings to published data for CMV. The study team found that while proliferative T-cells are present at baseline and during acute COVID-19 infection, they were not detected during the convalescent state. Similarly, resting, central memory, effector memory, and cytotoxic SARS-CoV-2-associated T-cells increase during acute infection and decrease by convalescence. However, some patients experience an increase in effector memory cells at recovery and those patients have gastrointestinal PASC symptoms.\(^{131}\) These findings suggest that gastrointestinal sequelae of PASC have a different kinetic pathway than other PASC sequelae.

The ISB study team also used the single cell RNAseq aggregate data to identify immune endotypes in COVID-19 patients. To do so, the study team separated immune responses into three categories: resting (or naïve-like), Th1-like, and Th2-like. Based on patients’ acute immune system response, the study team
was able to predict most patients’ convalescent immune states. However, the relationship between PASC and these states remains unclear. Patients with high levels of autoantibodies showed either a Th2-like immune response or an intermediate response between Th1- and Th2-like responses. Additionally, patients with strong viral signatures were intermediate between Th1- and Th2-like response. Dr. Heath emphasized that these findings suggest some of the heterogeneity observed in PASC reflects the diversity in traditional pathways that people take through disease and recovery.

**Co-Dependence between PASC Risk Factors**

Utilizing a multiomic dataset, the study team evaluated the relatedness between PASC risk factors over time. They found that relationships between PASC risk factors are lost over time, from COVID-19 diagnosis to convalescence. At diagnosis, there are several inter-relationships, particularly between autoantibodies, that align with different PASC risk factors (e.g., diabetes, EBV viremia). However, this inter-relatedness diminishes during acute infection and is gone by convalescence. Dr. Heath concluded that this finding both illustrates relationships between PASC factors and suggests a limited number of therapies are available for treating accumulated PASC factors; studying PASC only after acute infection provides a false impression that PASC factors are not related and therefore can be treated with multiple, general therapies.
The study team concluded that in the study population, EBV viremia, SARS-CoV-2 RNAemia in blood, and Type 2 diabetes each explained about 30% of PASC symptoms, whereas autoantibodies (at both clinical and subclinical levels) explained about 67% of PASC symptoms, suggesting that autoantibodies may be the most important PASC factor. These risk factors may overlap in patients with varied symptoms.

The observed temporal changes in the connections between PASC risk factors may also indicate that earlier intervention, when connections are stronger, may better impact the course and severity of PASC. For example, treating individuals for intensive care unit related delirium can offset the development of acute delirium. As another example, early treatment with antivirals may adjust the disease course not only for COVID-19 but also for PASC by addressing EBV viremia and SARS-CoV-2 RNAemia—two PASC risk factors.

Limitations of Findings
The findings of the ISB study have several limitations that may need to be addressed in future PASC research. The study followed only 300 COVID-19 patients for only 10-14 weeks. Type 2 diabetes may have been identified as a PASC risk factor due to the high incidence rate of this comorbidity in the study population. Other comorbidities (e.g., congestive heart failure) may be identified as PASC factors in larger cohort studies. The study measured only a limited number of autoantibodies, although the study team has now begun interrogating the patient population for autoantibodies against the full human proteome. Finally, the study was not sensitive to PASC from the Omicron variant, which may differ from previous PASC forms. The national RECOVER study, which will evaluate 17,000 patients over a 4-year period, may provide further explanation for other forms of PASC (e.g., cardiac PASC, circadian disruption). Dr. Heath also suggested that the global alterations in metabolism during acute COVID-19 infection challenge researchers’ ability to discern what may be important or relevant to PASC progression, but that longer-term follow-up studies like RECOVER may be able to provide insights.
Autoantibodies in COVID-19 and Other Infections

**PJ Utz, MD, Professor of Medicine and Associate Dean for Medical Student Research, Stanford University – Delivered March 9, 2022**

- Since early in the pandemic, COVID-19 infection has been associated with acute autoimmune and inflammatory manifestations (e.g., multisystem inflammatory disease in children, multisystem inflammatory disease in adults, severe arthralgias, Guillain Barre Syndrome).
- Autoantibodies and anti-cytokine autoantibodies (ACAs) are common in patients hospitalized with severe COVID-19, with some autoantibodies developing faster, suggesting a memory response based on a previous infection.
- Further research with larger cohorts is needed to determine the function of the autoantibodies/ACAs and whether they impact PASC development or severity.

**Introduction**

From the very beginning of the COVID-19 pandemic, physicians identified acute autoimmune and inflammatory manifestations associated with COVID-19 infection, including multisystem inflammatory disease in children (MIS-C), multisystem inflammatory disease in adults (MIS-A), severe arthralgias (e.g., resembling rheumatoid arthritis), and Guillain Barre Syndrome, among others. In addition, several published research papers noted that COVID-19 patients developed autoantibodies—including anti-nuclear antibodies (ANA), anti-neutrophil cytoplasmic antibodies (ANCA), anti-phospholipid antibodies (APLA), and multiple secreted protein targets of autoantibodies.\(^{38,136–138}\)

One study in 172 COVID-19 patients found that over half of them developed pathogenic APLA.\(^{41}\) The researchers found that the pathogenic APLA caused the formation of neutrophil extracellular traps (NETs), a mechanism by which neutrophils die that in COVID-19 patients is correlated with development of blood clots. When the research team purified IgG from patients with SARS-CoV-2 and added it to neutrophils, the neutrophils exploded and activated inflammatory pathways. The researchers also introduced IgG from COVID-19 patients and IgG from patients with catastrophic antiphospholipid syndrome (CAPS) into a mouse model and found that the IgG from both sets of patients caused similar blood clotting properties, with higher levels of antibodies in COVID-19 patients leading to more severe clotting. Researchers identified that the neutrophils in these NETs could be found in the clots, showing that NETs are driving the clotting process.

**Traditional Autoimmune Screening**

In response to these findings, Dr. Utz’s lab performed a study in hospitalized samples from patients with COVID-19 from March and April 2020 (when infections were all wild-type strain). The study had some limitations, including the inability to collect longitudinal samples for all patients, a lack of samples collected at admission to the hospital, a lack of age and sex matching among the healthy controls, and the
potential for elderly patients to present with autoantibodies not associated with COVID-19 infection.

First, the study team performed CLIA-certified assays to test patient samples for ANA and found that approximately 25% of patients had a positive ANA test. This small portion of positive tests differed from the findings cited in the literature that previously identified large numbers of patients with ANA. The researchers also used CLIA-certified assays to test for double stranded DNA antibodies and ANCA and, contrary to the literature, did not find many patients with autoantibodies that target the proteins myeloperoxidase (MPO) and proteinase 3 (PR3). Based on these findings, the study team concluded that common autoantigens (e.g., double-stranded DNA) are not prominent in COVID-19 patients and therefore screening these patients with traditional CLIA-certified assays would not identify the autoantigens targeted by the autoantibodies.

Autoantibodies in COVID-19
Based on this conclusion, the study team developed autoantigen microarrays traditionally used to characterize autoimmune diseases. Due to supply chain issues, the study team was limited to creating a 73-plex array. The study team also created a 41-plex cytokine microarray to measure patient response to cytokines and growth factors and a 73-plex COVID-19 viral array to test patient response against SARS-CoV-1, SARS-CoV-2, MERS, Ebola, and community coronaviruses. For all these arrays, the study team utilized the Luminex bead-based approach.
The study team found that autoantibodies are common in hospitalized patients with COVID-19. In their initial study and subsequent experiments, the team tested samples from over 500 patients hospitalized for COVID-19 at 8 centers in California, Pennsylvania, Illinois, and Germany. About half of the patients had antibodies against at least one autoantigen, versus approximately 5% of healthy controls. Additionally, approximately 75% of patients had at least one ACA. Autoantibodies identified include Beta 2 Glycoprotein 1, although this antigen is difficult to work with and therefore results are not conclusive. COVID-19 patients also had anti-C1q. C1q is a complement protein involved with removing immune complexes that are associated with inflammation and renal failure. Additionally, a small portion of patients had antibodies against ACE2. Many of the targeted antigens are commonly associated with rare connective tissue diseases (e.g., myositis) and are commonly found in the cytoplasm, as opposed to the nucleus. Furthermore, patients with these autoantibodies had strikingly high levels of them, well above the threshold of detection. Dr. Utz explained that this finding is significant because researchers have hypothesized that autoimmune myositis is triggered by acute infection. The research team also identified antibodies commonly associated with scleroderma, or systemic sclerosis. While the levels of these antibodies were not as high in COVID-19 patients as in prototype patients with limited scleroderma or diffuse systemic sclerosis, the findings were still abnormally high. Based on these findings, Dr. Utz proposed that a major outstanding question to be addressed will require epidemiology studies of PASC patients to determine if the incidence of scleroderma and myositis increases in the coming years, suggesting these diseases might be triggered by the virus. For example, Dr. Utz identified one COVID-19 patient from the Lambda interferon clinical trial with high levels of PM/Scl-75 antibodies, who the main study PI confirmed was the sickest of the entire cohort. Therefore, researchers have concluded that the patients developing autoantibodies are the patients who are most severely ill during acute COVID-19 infection.

A major outstanding question is whether these antibodies are newly formed in COVID-19 patients or are present prior to infection with SARS-CoV-2. To address this question, the study team compared patients who had longitudinal data and found that about 25% of them who did not have autoantibodies at baseline developed autoantibodies within 7 days and continued to have detectable autoantibodies 28 days later. For those autoantibodies developing quickly, researchers posit that there is a memory response enabling the quick rise to high levels in these patients. Additionally, some of these antigens appear to be newly induced.
Anti-Cytokine Antibodies in COVID-19

Researchers have found that some infectious diseases can actually be characterized as autoimmune diseases. While COVID-19 infection is itself harmful, a COVID-19 patient with ACA will have worse COVID-19 outcomes because those ACA block binding to cytokine receptors and prevent the immune system from fighting the infection. Numerous types of ACA have been identified (e.g., anti-erythropoietin, anti-interferon gamma) that affect individuals with autoimmune diseases. For example, patients with lupus have antibodies against interferon alpha, interleukin 2, and BAFF. However, recent research has also found ACAs that affect patients with severe COVID-19 infections. For example, one study found that 12% of men and 2-3% of women with severe COVID-19 infections had ACA against type 1 interferons. These results have been replicated via different methods in other studies. Researchers posit that the ACAs blocking interferon prevent immune system reactions and allow the virus to replicate more freely, leading to more severe disease.

When researchers tracked autoantibodies in COVID-19 patients over time, they found a mix that (1) were not present at baseline but increased over time, (2) were present at baseline and did not change, and (3) were present at baseline but disappeared over time. According to multiple studies, autoantibodies that develop after baseline are not limited to development early in hospitalization but may even develop more than 20 days after admission. Interferon epsilon, IL-17a, and IL-17f often develop early, with patients displaying high levels a week after initial infection. Even after the levels of any of these three classes of autoantibodies drop below the limit of detection via Luminex testing, some still have blocking capabilities. Dr. Utz noted that more research is needed to determine the function of the autoantibodies and whether they impact PASC development or severity.
Autoantibodies and Infections
Based on a query about whether similar autoantibody responses are seen with other infectious diseases, Dr. Utz’s lab reviewed pre-pandemic ICU patients to determine whether autoantibodies against cytokines are common in patients with severe disease. The study team found that healthy control patients did not have ACA, ICU patients without infection did not have many ACAs, but ICU patients with a variety of infections had a large number and variety of ACAs. These findings suggest that these patients with acute infections likely had underlying autoimmunity against these cytokines, which is modulating their response to the infections.

The study team also collected samples at Days 0, 7, and 28 from a cohort of patients with influenza, many of whom were critically ill. Some patients—particularly the most severely ill—had naturally occurring ACAs. One patient who developed very severe influenza and nearly died had particularly high levels of

Shaw, Clinical Infectious Diseases, clab1002, https://doi.org/10.1093/cid/clab1002
anti-interferon. The same patient later developed life-threatening COVID-19, despite being partially vaccinated. Additionally, the study team identified patients with new ACAs that act against traditional antigens (e.g., SRP54). Based on these results, Dr. Utz concluded that multiple viruses have the ability to trigger autoantibodies. Furthermore, Dr. Utz noted that previous influenza pandemics were associated with development of a PASC-like illness after acute infection—suggesting that the activation of the immune system and production of autoantibodies may play a role in these manifestations. The study team is continuing to perform research in this area. One finding of particular interest is that healthy patients who are vaccinated against COVID-19 do not develop autoantibodies. Researchers still need to determine the effect that vaccination would have on autoantibodies in individuals vaccinated after acute infection has already occurred.

The study team developed several cytokine receptor blocking assays that have been validated and are being used in their studies. The team has also identified a number of outstanding questions to address, including the prevalence of autoantibodies and ACA in COVID-19, whether they are transient or permanent, potential covariates (e.g., age, ethnicity), and how the autoantibodies relate to the subsets of PASC. Another outstanding question is whether the majority of COVID-19 patients developing autoantibodies have significant interstitial lung disease from their COVID-19 and whether the same autoantibodies correlate with the myositis and scleroderma autoantibodies, since myositis and scleroderma are both associated with lung fibrosis. Researchers require additional samples to answer some of these remaining questions, many of which may be addressed by the upcoming RECOVER study cohort.
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